Short Communication:
Limited Anti-HIV-1 Activity of Maraviroc in Mucosal Tissues

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Abstract

The potential of maraviroc (MVC), a small-molecule CCR5 antagonist, as a candidate to prevent HIV-1 sexual transmission by oral or topical dosing has not yet been completely established. Using relevant cellular and mucosal tissue explant models, we show partial antiviral activity of MVC when tested in multiple preclinical dosing strategies.

THE PREDOMINANT TRANSMISSION of R5 tropic HIV-1 isolates through sexual intercourse[1,2] makes CCR5 an appealing target to prevent viral entry in mucosal target cells. Maraviroc (MVC) is the first antiretroviral (ARV) small-molecule CCR5 inhibitor[3] to have been included in highly active antiretroviral therapy (HAART) and is currently being considered in the field of prevention as a candidate for oral pre-exposure prophylaxis (PrEP) or for topical application as a microbicide. Formulated as a microbicide gel, MVC has shown promising pharmacological results in humans and nonhuman primates (NHPs)[4,5] and efficacy in NHPs when tested as a vaginal gel.[6,7]

To model different potential prevention dosing conditions, we tested drug efficacy before viral challenge (3-h drug exposure), before and after challenge (24-h drug exposure), or continuous drug exposure mimicking sustained release. We first assessed the activity of MVC with these dosing conditions in activated peripheral blood mononuclear cells (PBMCs) and other cellular models for preclinical evaluation in a mucosal environment, including immature dendritic cells (iDCs) and monocyte-derived macrophages (MDMs).[8] We observed different susceptibility to infection in the three models with higher levels of p24 in culture supernatant after 14 days of culture in PBMCs (33.29 ± 1.43 ng/ml) compared with MDMs (12.77 ± 0.73 ng/ml) and iDCs (1.27 ± 0.60 ng/ml).

With all three drug exposure times, a dose–response curve was obtained against HIV-1 BaL in activated PBMCs (Fig. 1a) and MVC was able to reach an IC_{50} at low and decreasing nanomolar concentrations with increasing dosing times (9.93 ± 1.68 nM with a 3-h exposure, 5.00 ± 1.38 nM with a 24-h exposure, and 0.94 ± 0.12 nM after continuous exposure). The IC_{50} of MVC in monocyte-derived iDCs against HIV-1 BaL was in the nanomolar range as shown in PBMCs. The

antiviral potency of MVC increased with longer drug exposure times, resulting in a reduction of the IC_{50} value from 23.96 ± 26.08 nM with a 3-h exposure to 3.37 ± 2.21 nM with a 24-h exposure and 0.47 ± 0.14 nM with continuous exposure.

When iDCs were pre-exposed to MVC for 3 h, only partial inhibition was observed, failing to reach 95% inhibition even at the highest drug concentration tested ($10\,\mu M$) (Fig. 1b). The plateau of maximum inhibition was increased with longer drug exposure, 24 h, and continuous exposure and was reached at lower concentrations with IC_{95} values of 68.44 ± 48.85 nM and 20.91 ± 23.42 nM, respectively.

The level of CCR5 expressed on the surface of MDMs has been described to increase when generated in the presence of granulocyte-macrophage colony-stimulating factor and to be higher than that measured in monocyte-derived DCs.[9] The expression of higher levels of CCR5 on the cell surface requires higher doses of MVC to prevent HIV-1 entry[10] (C. Herrera and R.J. Shattock, unpublished data); hence, we assessed the potency of MVC in MDMs with the more active exposure duration mimicking sustained release. With continuous exposure during 14 days, MVC was only able to reach ∼80% of inhibition at $10\,\mu M$, the highest concentration tested against HIV-1 BaL (Fig. 1c). The IC_{50} value in these conditions was 112.23 ± 67.45 nM.

Based on the results obtained with monocyte-derived cells, we then titrated the activity of MVC in mucosal tissue explants, which better model the environment of the portal of viral entry during sexual transmission. Surgically resected ectocervical and colorectal tissues were used to assess the activity of the drug in the female reproductive compartment and in the sigmoidal tract, respectively, using the same three dosing times tested in the cellular models (3-h exposure, 24-h exposure, and continuous exposure).

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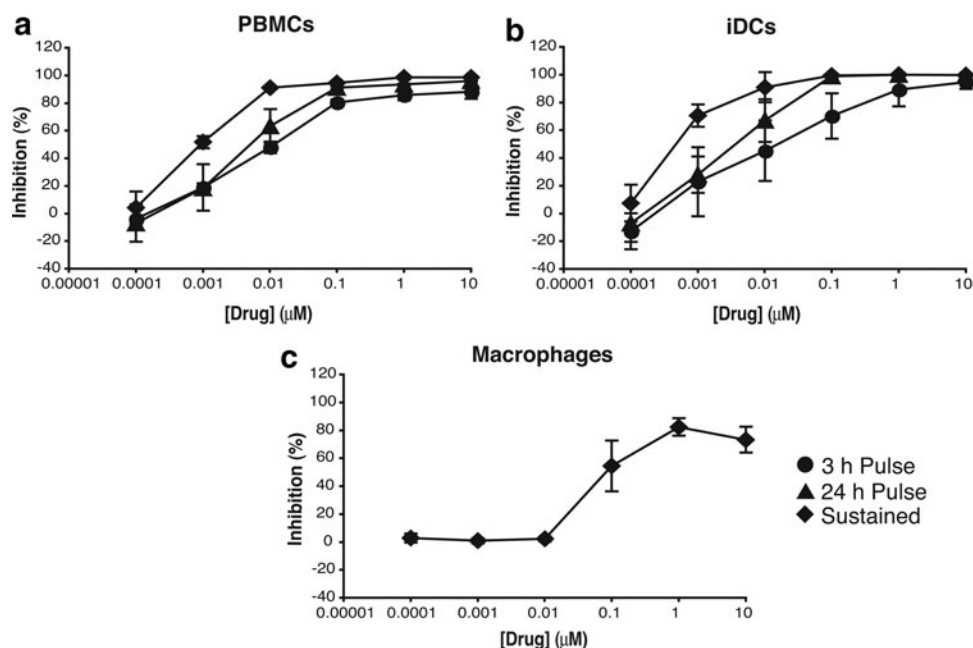
FIG. 1. (a) PBMCs were isolated from multidonor buffy coats from healthy HIV-seronegative donors by centrifugation onto Ficoll-Hypaque, mitogen stimulated as previously described,[27] and maintained in RPMI 1640 medium containing 10% FCS, 2 mM L-glutamine, antibiotics (100 U of penicillin/ml, 100 μg of streptomycin/ml), and 100 U of IL-2/ml. (b) iDCs were grown from PBMC-derived monocytes cultured for 6 days in complete RPMI medium supplemented with 1000 U/ml GM-CSF and 500 U/ml IL-4 (R&D Systems). iDCs were phenotypically characterized by staining with anti-CD40, anti-CD80, anti-CD86, anti-CD83, anti-CD209, anti-CD123, and anti-CD11c (BD Pharmingen). (c) MDMs were obtained from buffy coat-derived PBMCs by adherence and matured for 5–7 days by culture in RPMI 1640 plus 10% human serum (HS) and 20 ng/ml of GM-CSF (R&D Systems). Cells were exposed to serial dilutions of MVC for 1 h before addition of HIV-1 BaL at 2,000 TCID$_{50}$/ml. After 2 h of incubation, cells were washed and cultured for 14 days in the absence (3-h exposure, ●) or presence of MVC for a total of 24 h (24-h exposure, ▲) or the duration of the assay (continuous exposure, ◆). The concentrations of p24 in harvested supernatants were quantified by ELISA. The percentage of inhibition was normalized relative to the p24 values obtained for cells not exposed to virus (0% infectivity) and for cells infected with virus in the absence of compound (100% infectivity). Data are means ± standard deviations from three independent experiments performed in triplicate. FCS, fetal calf serum; GM-CSF, granulocyte-macrophage colony-stimulating factor; iDCs, immature dendritic cells; IL, interleukin; MDM, monocyte-derived macrophage; MVC, maraviroc; PBMC, peripheral blood mononuclear cell.

Interestingly, no significant antiviral activity was observed for MVC with any of the dosing strategies in ectocervical tissue even at the highest concentration of MVC tested (Fig. 2a). However, dose–response curves for MVC (Fig. 2b) were obtained when ectocervical migratory cells, which have been characterized as DCs,[11] were harvested from MVC-treated and HIV-1-challenged tissue and cocultured with CD4$^+$ T PM-1 cells. This model mimics the potential cell-associated transmission of HIV-1 from virus-exposed iDCs to uninfected CD4$^+$ T cells that occurs during the local expansion of infection following establishment of the initial foci of infection in mucosal tissues.[12,13]

Similar to the results obtained with iDCs, the potency of MVC increased with longer time of exposure, reaching higher levels of inhibition with 24-h and continuous exposure than with a 3-h exposure (Fig. 2b). This increase in activity was also reflected in a decrease of the IC$_{50}$ value for MVC from 12.06 ± 7.36 nM with a 3-h exposure to 1.86 ± 0.87 nM with a 24-h exposure and to 0.49 ± 0.56 nM with continuous exposure. The IC$_{50}$ values obtained for each dosing protocol were in the same range as those observed for the same time of exposure in iDCs.

To investigate if the lack of activity of MVC in ectocervical explants was due to the low number of activated CCR5$^+$ cells

in the female genital tract compared with the colorectal mucosa,[14–16] we compared the antiviral activity of MVC in nonstimulated versus phytohaemagglutinin (PHA) and interleukin (IL)-2 pretreated tissue explants from the same donors.

As expected, the levels of p24 measured in culture supernatants of stimulated ectocervical tissue (1.69 ± 1.34 ng/ml) were greater than in nonstimulated tissue (0.54 ± 0.19 ng/ml). However, this increase was not observed in cocultures of cervical migratory cells with PM-1 cells (nonstimulated: 252.65 ± 241.14 ng/ml; and stimulated: 336.39 ± 218.52 ng/ml). With continuous exposure to MVC, a dose–response curve against HIV-1 BaL was observed with stimulated ectocervical tissue (Fig. 2c), reaching ∼80% of inhibition at the highest concentration of MVC tested. The IC$_{50}$ value for MVC in these conditions was 4.97 ± 2.57 nM. Stimulation of ectocervical explants did not increase the inhibitory potency of MVC in the migratory *trans*-infection assays (data not shown).

CCR5 is a highly modulated G-protein-coupled receptor (GPCR) on the cell surface and can be expressed with different states of activation, as a monomer, as a homodimer, or can even form heterodimers with other GPCRs.[17–19] These results suggest that the lack of antiviral activity of MVC in nonstimulated ectocervical tissue could be due not only to a
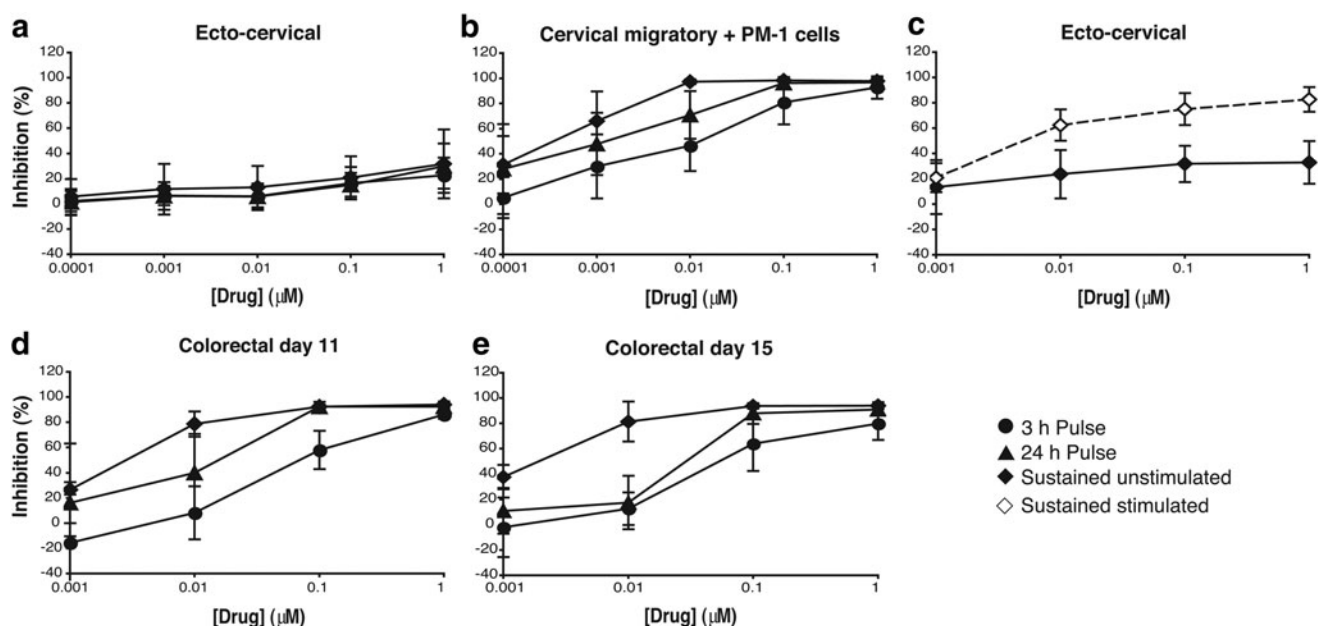
FIG. 2. **(a–c)** Ectocervical tissue was obtained from premenopausal females undergoing routine hysterectomies with nonmalignant pathology. Tissue was dissected into explants as previously described[11] and incubated with serial dilutions of MVC for 1 h before virus (10^4 $TCID_{50}$/ml) was added for 2 h. Explants were then washed four times with phosphate-buffered saline and cultured in fresh plates **(a)** or migratory cells were harvested at 24 h postinfection and cocultured with 40,000 PM-1 cells **(b)**. Both culture models were kept for 15 days in RPMI 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics (100 U of penicillin/ml, 100 μg of streptomycin/ml) in the absence (3-h exposure, ●) or presence of MVC for a total of 24 h (24-h exposure, ▲) or the duration of the assay (continuous exposure, ◆). **(c)** Stimulated ectocervical explants were treated with 100 U/ml IL-2 and 5 μg/ml PHA for 72 h before infection, with all cultures subsequently maintained in 100 U/ml IL-2. Stimulated (◇) and non-stimulated (◆) explants were exposed continuously to MVC. **(d, e)** Colorectal tissue was obtained from patients undergoing rectocele repair and colectomy from colorectal cancer. Colorectal explants were maintained with Dulbecco's modified Eagle's medium containing 10% FCS, 2 mM L-glutamine, and antibiotics (100 U of penicillin/ml, 100 μg of streptomycin/ml, 80 μg of gentamicin/ml). Tissue explants were treated and exposed to virus as the ectocervical explants. After four washes, explants were transferred onto gelfoam rafts (Welbeck Pharmaceuticals) and cultured for 15 days in the absence (3-h exposure, ●) or presence of MVC for a total of 24 h (24-h exposure, ▲) or the duration of the assay (continuous exposure, ◆). Antiviral activity was assessed at days 11 **(d)** and 15 **(e)** of culture. For all tissues, ∼50% of the supernatants were harvested every 2–3 days and explants were refed with fresh media with or without MVC. For all assays, p24 concentrations in harvested supernatants were quantified by ELISA. The percentage of inhibition was normalized as described in Figure 1. Data are means ± standard deviations from three independent experiments performed in triplicate. All tissues were collected after receiving signed informed consent from all patients and under protocols approved by the Local Research Ethics Committee. All patients were HIV negative. PHA, phytohaemagglutinin.

low level of CCR5$^+$ cells but also to their resting state where CCR5 may be expressed in different conformations to that on PHA/IL-2-stimulated cells.

We next assessed the antiviral activity of MVC in colorectal tissue explants. The titration of MVC with a 3-h exposure and after 15 days of culture resulted in a dose–response curve with an IC_{50} of 109.88 ± 84.51 nM and maximum inhibition below 80% (with an IC_{70} of 238.20 ± 85.24 nM) (Fig. 2e). This plateau as well as the potency of MVC was progressively increased with longer periods of tissue exposure to the drug (Fig. 2e). The IC_{50} values decreased to 22.05 ± 13.75 nM after a 24-h exposure and to 2.18 ± 1.05 nM with continuous exposure.

Interestingly, in this model, the maximum antiviral potency of MVC was more pronounced at an earlier time point in culture, day 11 (Fig. 2d), with a 3-h exposure, displaying a maximum level of inhibition above 85%, with an IC_{50} of 79.66 ± 50.95 nM and an IC_{70} of 162.76 ± 72.79 nM compared with the day 15 IC_{50} of 109.88 ± 84.51 nM and IC_{70} of 238.20 ± 85.24 nM described

above. When comparing the levels of p24 in culture supernatant, as a result of a productive infection, there was an increase of p24 between days 11 (4.31 ± 2.48 ng/ml) and 15 (18.84 ± 5.74 ng/ml). Independently of the time point analyzed, day 11 or 15, the inhibitory concentrations observed during sustained exposure of colorectal tissue tended to be 1 log higher than those observed in ectocervical tissue.

Histological analysis has shown that colorectal explants remained viable for more than 10 days with progressive loss of architecture, but maintenance of CD4:CD8 T-cell ratios.[20] There is currently a paucity of data regarding preservation of functions such as immune competence and mucus secretion for tissue explants; however, conservation of function has been described in other tissue explants.[21] Validation of the explant model with respect to intracellular drug distribution is not yet available. However, in combination with other models, such as TZM-bl cells and blood derived-cells used in this study, it provides powerful preclinical information to identify efficacious prevention candidates.

The differential activity of MVC in the cellular and tissue explant models likely reflects heterogeneity in CCR5 conformation and/or expression.[14–16] CCR5 is a dynamic receptor, where abundance and spatial distribution are modulated by binding of natural ligands and derivatives, inducing oligomerization and endocytosis,[22,23] and by cellular activation, leading to enhanced expression.[24,25] The binding of HIV gp120 to CCR5, and therefore susceptibility to infection, is critically affected by the coreceptor heterogeneity and conformation.[22,26,27]

Crystallographic studies[17,28] have revealed that gp120 and MVC do not bind to the same structural determinants in CCR5, but that allosteric modulation of CCR5 by MVC can inhibit gp120 binding, preventing infection.[17] However, MVC preferentially binds to higher energy conformations of CCR5,[29] displaying differential anti-HIV activity against G-protein-coupled and uncoupled forms.[30] The comparative lack of antiviral activity of MVC in nonstimulated ectocervical tissue compared with PHA/IL-2-activated cultures suggests that resting tissue expresses CCR5 in a conformation that may be refractory to MVC binding and/or allosteric modulation required to prevent HIV infection.[17] This important observation warrants further investigation, but this falls beyond the scope of this current study. Furthermore, we cannot exclude that differences in the activity of drug transporters between the two mucosal compartments (rectal and cervical) might also impact on MVC activity[31,32]

The impact of differential states of target cell activation in specific mucosal tissues has also been observed for other PrEP agents such as reverse transcriptase inhibitors (RTIs). In NHPs, despite similar levels of drug found in blood, rectal, and lymphoid tissues, no protection was observed following rectal challenge due to higher levels of CD4 T-cell activation in rectal tissue compared with other compartments.[33] In this study, cellular activation causes increase of intracellular nucleotides, which are the natural substrates of RT, therefore, competing with RTIs. While the mechanisms are distinct, differences in the activation status of HIV target cells within the different mucosal compartments (vaginal and rectal) likely impact on the activity of MVC through modulation of CCR5 expression and/or conformation as described above. These data highlight the need to take into account the activation status and target cell population within different tissue microenvironments when evaluating drugs for mucosal HIV prophylaxis.

The partial activity of MVC particularly in ectocervical tissue raises significant questions over its use as a stand-alone prevention modality. In fact, no protective activity has been observed when MVC has been tested as an oral PrEP candidate in a study in NHPs[34] and in the first two clinical trials conducted to date.[35,36] Furthermore, the duration of protection in NHPs following vaginal application was short.[7] Nevertheless, the mechanism of action and pharmacological profile[37] of MVC make this compound a good candidate for the design of an ARV combination-based prevention strategy, as reported in an NHP study where a combination of MVC with tenofovir, an RTI, was highly protective against rectal challenge when formulated as a gel microbicide.[38] The ongoing oral PrEP clinical trial, HPTN 069/ACTG 5305 (NEXT-PrEP), evaluating double combinations of MVC, FTC, and TDF will be key to inform the potential of MVC in the field of prevention.

Author Disclosure Statement

No competing financial interests exist.

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