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Cellulose Acetate 1,2-Benzenedicarboxylate Inhibits Infection by Cell-Free and Cell-Associated Primary HIV-1 Isolates

HONG LU¹, QIAN ZHAO¹, GREG WALLACE², SHUWEN LIU¹, YUXIAN HE¹, ROBIN SHATTOCK², A. ROBERT NEURATH¹, and SHIBO JIANG¹

¹ Lindsley F. Kimball Research Institute, New York Blood Center, New York, New York 10021

² St. George's Hospital Medical School, University of London, Tooting, London SW17 0RE, United Kingdom

Abstract

Cellulose acetate 1,2-benzenedicarboxylate (CAP), a pharmaceutical excipient used for enteric film coating of capsules and tablets, was previously shown to have potent inhibitory activity against infection by human immunodeficiency virus type 1 (HIV-1) T cell line-adapted (TCLA) strains. In the present study, we determined the inhibitory activity of CAP against infection by cell-free and cell-associated primary HIV-1 isolates with distinct genotypes and biotypes in cervical explants, peripheral blood mononuclear cells (PBMCs), monocyte-derived macrophages (MDMs), and CEMx174 5.25M7 cells. CAP blocked infection by cell-free and cell-associated HIV-1 in cervical explants. It inhibited infection by cell-free primary HIV-1 isolates (clades A to G and group O) in PBMCs, MDMs, and CEMx174 5.25M7 cells and blocked transmissions of the cell-associated primary HIV-1 isolates from dendritic cells (DCs) to PBMCs, from MDMs to PBMCs, and from PBMCs to CEMx174 5.25M7 cells. The inhibitory activity of CAP on infection by the cell-free and cell-associated primary HIV-1 isolates is independent of viral subtypes and coreceptor usage. These data suggest that CAP is a good microbicide candidate that can be further developed for preventing sexual transmission of HIV-1.

INTRODUCTION

Sexual transmission is the most common route of spread of human immunodeficiency virus (HIV)^{1,2} and more than 90% of adolescent and adult HIV infections worldwide have resulted from heterosexual intercourse.³ Both cell-free and cell-associated viruses present in genital secretions can be sexually transmitted.^{4–6} Therefore, development of topically applied, safe, potent, and affordable microbicides is urgently needed for prevention of sexual transmission of HIV-1.

Cellulose acetate 1,2-benzenedicarboxylate (CAP) is a pharmaceutical excipient with a long history of safe use in humans as an enteric film coating polymer for capsules and tablets. It inhibits infection by HIV type 1 (HIV-1) through multiple mechanisms of action, including inactivation of virions and inhibition of virus entry by blocking gp120 binding to the coreceptor (CXCR4 or CCR5) and by inducing “dead-end” gp41 six-helix bundle formation.^{7–9} It has significant synergistic anti-HIV-1 activity with reverse transcriptase inhibitors (RTIs).¹⁰ CAP blocks infection via vaginal challenge by simian immunodeficiency virus (SIV) and SIV/HIV chimeric viruses (SHIV) in rhesus macaque and pigtail macaque monkeys.^{11–13} CAP also has

Address reprint requests to: Shibo Jiang, Lindsley F. Kimball Research Institute, New York Blood Center, 310 E. 67th Street, New York, NY 10021, sjjiang@nybloodcenter.org.

microbicidal activity against a broad spectrum of sexually transmitted disease (STD) pathogens, including herpes virus types 1 (HSV-1) and 2 (HSV-2), cytomegalovirus, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Haemophilus ducreyi*, *Chlamydia trachomatis*, *Treponema pallidum*, and bacteria associated with bacterial vaginosis, but does not affect the viability of lactobacilli, required for maintaining a healthy vaginal environment.^{7,14} CAP has low or no *in vitro* cytotoxicity.¹⁵

Although the inhibitory activity of CAP on infection by T cell line-adapted (TCLA) HIV-1 strains has been well proven,^{7–10} only a few primary HIV-1 isolates have been tested for its *in vitro* efficacy.¹⁶ It is essential to further determine its antiviral activity against infection by cell-free and cell-associated primary HIV-1 isolates with distinct genotypes and biotypes. Here we demonstrate that CAP effectively inhibited infection by a broad spectrum of primary HIV-1 isolates in peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs), which are the primary target cells for HIV-1, and CEMx174 5.25M7 cells, a cell line expressing CD4 and both coreceptors (CXCR4 and CCR5). CAP also blocked transmission of cell-associated primary HIV-1 isolates from dendritic cells (DCs) to PBMCs, from MDMs to PBMCs, and from PBMCs to CEMx174 5.25M7 cells. These results suggest that CAP is effective in inhibiting infection by both cell-free and cell-associated primary HIV-1 isolates with distinct genotypes and biotypes. Therefore, it has great potential for use as a microbicide for the prevention of sexual transmission of HIV-1.

MATERIALS AND METHODS

Reagents

CAP was dissolved in 30 mM sodium acetate buffer (pH 5.9) to 30 mg/ml as a stock solution and stored at 4°C (no more than 6 weeks). The working solutions of CAP were freshly prepared by diluting the stock solution in culture medium. HIV-1 primary isolates and anti-p24 monoclonal antibody (183-12H-5C) were obtained from the NIH AIDS Research and Reference Reagent Program (Bethesda, MD). HIV-1 strain BaL was obtained from the AIDS Reagent Project (National Institute for Biological Standards and Control, Potters Bar, UK). The lymphoid cell line CEMx174 5.25M7, kindly provided by N.R. Landau through C. Cheng-Mayer, is stably transduced with an HIV-1 long terminal repeat (LTR)–green fluorescent protein (GFP) reporter and luciferase reporter construct and expresses CD4 and both coreceptors, CXCR4 and CCR5.¹⁷ These cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), puromycin (1 µg/ml), and G418 (200 µg/ml).

Infection of PBMCs by primary HIV-1 isolates

The inhibitory activity of CAP on infection of PBMCs by primary HIV-1 isolates was determined as previously described.¹⁸ PBMCs were isolated from white cell concentrates, which were separated from the blood of health donors at the New York Blood Center (New York, NY) by standard density gradient centrifugation with Histopaque-1077 (Sigma, St. Louis, MO). The cells were plated in a 75-cm² plastic flask and incubated at 37°C for 2 hr. The nonadherent cells were collected (the adherent cells would be harvested for isolation of monocytes and dendritic cells as described below) and resuspended at 5×10^6 /ml in 10 ml of RPMI 1640 medium containing 10% FBS, phytohemagglutinin (PHA, 5 µg/ml; Sigma), and interleukin 2 [(IL-2) 100 U/ml; Sigma], followed by incubation at 37°C for 3 days. The PHA-stimulated PBMCs were collected, washed, and resuspended in culture medium to 1×10^5 /ml. Fifty microliters of CAP at graded concentration was incubated with an equal volume of a primary HIV-1 isolate at a multiplicity of infection (MOI) of 0.01 at 37°C for 30 min, followed by addition of 100 µl of PHA-stimulated PBMCs (5×10^5 /ml). After incubation at 37°C overnight, the culture supernatants were replaced with fresh medium without CAP, and then

the medium was changed twice per week. On day 7 postinfection, 100- μ l aliquots of culture supernatants were collected from each well, mixed with equal volumes of 5% Triton X-100, and assayed for p24 antigen by an enzyme-linked immunosorbent assay (ELISA) as previously described.¹⁸ The percent inhibition of p24 production and the effective concentration for 50% inhibition (EC_{50}) were calculated with the computer program Calcsyn,¹⁹ kindly provided by T. C. Chou (Sloan-Kettering Cancer Center, New York, NY).

Infection of monocyte-derived macrophages by primary HIV-1 isolates

MDMs were isolated from PBMCs and infected with HIV-1 as previously described.²⁰ Briefly, the adherent cells harvested from PBMCs were plated in 100-mm-diameter tissue culture dishes (5×10^7 cells per dish) and cultured in RPMI 1640 medium supplemented with 5% human serum, penicillin (100 U/ml), and streptomycin (100 μ g/ml) at 37°C for 1 week. The cells were maintained in the same RPMI 1640 medium plus macrophage colony-stimulating factor (GM-CSF, 100 U/ml; Immunex, Seattle, WA) for an additional 1 week to allow differentiation of monocytes into MDMs. For inhibition of HIV-1 infection, 100 μ l of MDMs at 1×10^6 /ml in culture medium (RPMI 1640 containing 5% human serum) was infected with 50 μ l of an HIV-1 isolate (MOI of 0.1) in the presence of CAP at graded concentration. After culture at 37°C overnight, the culture medium was replaced with fresh culture medium without CAP. The culture supernatants were collected 7 days postinfection for measuring p24 by ELISA. The percent inhibition of p24 production and the EC_{50} values were calculated as described above.

HIV-1 infection of CEMx174 5.25M7 cells

To determine the inhibitory activity of CAP on infection by primary HIV-1 isolates in CEMx174 5.25M7 cells, 50 μ l of CAP at graded concentration in triplicate was incubated with an equal volume of a primary HIV-1 isolate at an MOI of 0.01 at 37°C for 30 min, followed by addition of 100 μ l of CEMx174 5.25M7 cells (5×10^5 /ml). After incubation at 37°C overnight, the culture medium was replaced with fresh medium without CAP. On day 3 postinfection, the cells were harvested and lysed for analysis of luciferase activity, using a luciferase assay kit (Promega, Madison, WI) and a luminometer (model Ultra 386; Tecan, Durham, NC) according to the manufacturer's instructions. The percent inhibition of luciferase activity and the EC_{50} values were calculated as described previously.¹⁸

HIV-1 infection of cervical explants

Cervical explants were obtained (with consent and local IRB approval) from premenopausal women undergoing planned therapeutic hysterectomy at St. George's Hospital (London, UK). Semen samples were obtained from normal donors (with consent and local IRB approval) at St. George's Hospital Medical School. Intact cervical explants (3 mm), consisting of both epithelial and stromal tissue, were cultured in Eagle's minimal essential medium (EMEM) supplemented with penicillin, streptomycin, L-glutamine, and HEPES buffer (Sigma) on squares of stainless-steel mesh, individually, in 24-well flat-bottomed plates at 37°C in a humidified atmosphere containing 5% CO₂ as previously described.²¹ To determine the inhibitory activity of CAP on infection of explants by cell-free virus, explants were exposed to 100 μ l of HIV-1_{BaL} (103 TCID₅₀/explant) diluted in culture medium or in 25% semen in the absence or presence of CAP at 37°C for 2 hr. In some experiments, CAP was added to the explants 1 hr after viral inoculation. After four washes with 200 μ l of PBS, explants were cultured for 10 days before collection of the supernatants for measuring p24 antigen by ELISA. ²¹ To determine whether CAP interacts with the target cells in the explants or with virus, the explants were pretreated with CAP before addition of virus (1 hr at 37°C followed by four washes with PBS) or virus was pretreated with CAP (1 hr at 37°C by centrifugation at $12,000 \times g$ for 2 hr and two washes with PBS) prior to inoculation of explants. For determining the inhibitory activity of CAP on infection of explants by cell-associated virus, PHA (5 μ g/ml)-

and IL-2 (10 $\mu\text{g/ml}$) stimulated PBMCs (1×10^6) were infected with HIV-1_{BaL} (103 TCID₅₀) for 7 days, followed by extensive washes with PBS. Chronically infected cells were treated with mitomycin C (200 $\mu\text{g/ml}$; Sigma) for 1 hr at 37°C prior to use. The HIV-1-infected PBMCs were cocultured with explants in the absence or presence of CAP (1000 $\mu\text{g/ml}$) at 37°C for 2 hr. After extensive washes, explants were cultured for 10 days. Subsequently, supernatants were collected for measuring p24 antigen production.

Isolation and identification of DCs

Blood DCs were obtained from healthy blood donors (New York Blood Center), using the procedures previously described.^{22–24} The nonadherent cells were removed by washing four times with warm RPMI 1640. The adherent cells were cultured with RPMI 1640 medium containing IL-4 (1000 U/ml; R&D Systems, Minneapolis, MN), GM-CSF (1000 U/ml; Immunex) and 5% heat-inactivated human AB serum. Cytokines were replenished on alternate days. On day 6, lipopolysaccharide (LPS, Sigma) was added to the culture medium (20 ng/ml) to induce DC maturation. On day 8, the mature DCs were harvested and analyzed by one-color flow cytometry using fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibody against human CD3, CD4, CD14, CD19, CD83, CD86, HLA-DR, and DC-SIGN, and isotype controls (all from BD Biosciences Pharmingen, San Diego, CA) as previously described.²⁴ Briefly, cells were resuspended in Hanks' balanced salt solution (HBSS; Gibco Invitrogen, Carlsbad, CA) and incubated for 15 min with antibodies (5 $\mu\text{g/ml}$) at room temperature. After washing twice with HBSS, the cells were fixed with 1% formaldehyde, and analyzed with a FACScan flow cytometer and CellQuest software (BD Biosciences Immunocytometry Systems, San Jose, CA).

HIV-1 transmission from DCs to PBMCs

Mature DCs were exposed to a primary HIV-1 isolate at an MOI of 0.01 at 37°C overnight and washed three times to remove unbound virus. The cells were treated with mitomycin C (200 $\mu\text{g/ml}$; Sigma) for 1 hr at 37°C.²¹ The HIV-1-pulsed DCs were cultured with CAP at graded concentrations at 37°C for 30 min before addition of PBMCs. After incubation at 37°C overnight, the culture medium was replaced with fresh culture medium containing no CAP. On day 7 after coculture, virus replication in PBMCs was monitored by measuring p24 production.

HIV-1 transmission from MDMs to PBMCs

MDMs were infected by a primary HIV-1 isolate (MOI, 0.1) for 14 days as described above and washed twice with RPMI 1640 medium. The HIV-1 infected MDMs resuspended in RPMI 1640 medium supplemented with 5% human serum ($2 \times 10^5/\text{ml}$) were cultured at 37°C for 2 hr with PHA- and IL-2-stimulated PBMCs ($5 \times 10^5/\text{ml}$) from the same donor in the absence or presence of CAP at graded concentration. The non-adherent PBMCs were then transferred to a new plate and incubated at 37°C for 7 days. The culture supernatants were collected for measuring p24 by ELISA as described above.

HIV-1 transmission from PBMCs to CEMx174 5.25M7 cells

PHA-stimulated PBMCs were infected by HIV-1 (MOI, 0.01) for 7 days as described above. After washes, 50 μl of HIV-1-infected PBMCs ($1 \times 10^5/\text{ml}$) were incubated with 50 μl of CAP at graded concentration at 37°C for 30 min. Then, 100 μl of CEMx174 5.25M7 cells (2×10^5) was added and cocultured at 37°C for 3 days. The cells were collected and lysed for analysis of luciferase activity, using a luciferase assay kit (Promega) as described above.

RESULTS

CAP inhibits infection of cervical explants by cell-free and cell-associated HIV-1

Heterosexual transmission of HIV-1 from men to women is initiated by HIV-1 infection of cells (e.g., dendritic cells, macrophages, and T lymphocytes) in the vaginal and cervical mucosa. Therefore, vaginal and cervical mucosa explants can be used as an *in vitro* model for evaluation of anti-HIV agents against mucosal HIV-1 infection.²⁵ Using such model, we tested the inhibitory activity of CAP on infection of cervical explants by cell-free and cell-associated HIV-1. As shown in the Fig. 1, cell-free HIV-1_{BaL} (R5 virus) in medium with or without 25% human semen could replicate in the cervical explants. CAP dissolved in medium and medium containing 25% human semen, when added simultaneously with the virus, inhibited HIV-1 replication in cervical explants. However, the inhibitory effect of CAP decreased significantly if added to cervical explants 1 hr after virus challenge. Explants pretreated with CAP were susceptible to HIV-1 infection, but virus pretreated by CAP lost infectivity. These results confirm that CAP is not targeted to the receptor or coreceptors on the cells, but interacts with envelope glycoproteins on the virions responsible for virus entry or directly inactivates virus.⁷⁻⁹ Cell-associated HIV-1_{BaL} could be efficiently transmitted from infected PBMCs to cervical explants and CAP was effective in blocking transmission of cell-associated HIV-1.

CAP inhibits infection by primary HIV-1 isolates of MDMs, PBMCs, and 5.25M7 cells

Both MDMs and CD4⁺ T lymphocytes in PBMCs are the primary target cells for HIV-1 infection. Here we show that CAP inhibited, in a dose-dependent manner, infection of both MDMs and PBMCs by all the primary HIV-1 isolates tested (Fig. 2A and B, and Table 1). The EC₅₀ values for inhibition of HIV-1 infection in MDMs and PBMCs ranged from about 1 to 100 µg/ml. However, we could not find a correlation between the sensitivity of the HIV-1 isolates to CAP and their subtypes or coreceptor usage. Using CEMX174 5.25M7 cells, we were able to measure the infectivity of the primary HIV-1 isolates.¹⁸ Here we determined the inhibitory activity of CAP on infection by primary HIV-1 isolates of CEMX174 5.25M7 cells. The results indicated that CAP inhibited infection of CEMX174 5.25M7 cells by all primary HIV-1 isolates tested, consistent with the results obtained with PBMCs (Fig. 2C and Table 1). Interestingly, the EC₅₀ values are about 3- to 20-fold lower than those obtained by using PBMCs, suggesting that the assay system using CEMX174 5.25M7 cells is more sensitive than that using PBMCs for determining the inhibitory activity of compounds against primary HIV-1 isolates.

CAP inhibits transmission of cell-associated primary HIV-1 isolates from DCs to PBMCs, from MDMs to PBMCs, and from PBMCs to 5.25M7 cells

It was reported that CAP blocked HIV-1 infection of human immature Langerhans cells within epidermal epithelial explants.²⁶ However, mature DCs do not support HIV-1 replication but efficiently transmit HIV-1 to T cells.^{24,27} To determine whether CAP inhibits HIV-1 transmission from mature DCs to PBMCs, adherent mononuclear cells were isolated from PBMCs, cultured in the presence of IL-4, and stimulated with LPS to induce maturation of DCs. As analyzed by flow cytometry using a series of mAbs, the cells express low levels of CD3, CD4, CD14, and CD19, and high levels of CD83, CD86, and DC-SIGN (data not shown), suggesting that these cells are mature DCs. Consistently with the earlier reports,^{24,27} no HIV-1 production in mature DCs was detected 12 days after inoculation with primary HIV-1 isolates. However, HIV-1 replication was detected in PBMCs that were cocultured with the HIV-1-pulsed DCs (data now shown). After addition of CAP to the coculture of HIV-1-pulsed DCs with PBMCs, replication of primary HIV-1 isolates in PBMCs was inhibited by CAP in a dose-dependent manner (Fig. 3A and Table 2), indicating that CAP is effective in blocking HIV-1 transmission from DCs to PBMCs. Similarly, no significant HIV-1 replication was observed in PBMCs that were cocultured with HIV-1-infected MDMs (Fig. 3B and Table 2), indicating

that CAP blocks HIV-1 transmission from MDMs to PBMCs. To determine whether HIV-1 could be transmitted from PBMCs to CEMx174 5.25M7 cells, which express CD4 receptors and both CXCR4 and CCR5 coreceptors, PBMCs infected with an HIV-1 primary isolate were cocultured with CEMx174 5.25M7 cells in the presence of CAP at graded concentrations. After 3 days, the level of luciferase activity, representing HIV-1 infectivity in CEMx174 5.25M7 cells, was measured. As shown in Fig. 3C and Table 2, CAP blocked transmission of primary HIV-1 isolates from PBMCs to CEMx174 5.25M7 cells, confirming that CAP can prevent transmission of cell-associated primary HIV-1 isolates.

DISCUSSION

An ideal topically applied microbicide should be effective against infection by both cell-free and cell-associated primary HIV-1 isolates. Previously, we demonstrated that CAP inhibited *in vitro* infection by several cell-free TCLA HIV-1 strains.⁷ In the present study, we have shown that CAP blocks *ex vivo* infection by both cell-free and cell-associated TCLA HIV-1 strains in cervical explants (Fig. 1). However, there are insufficient data to prove that CAP is also effective in blocking infection by both cell-free and cell-associated primary HIV-1 isolates. This is a critical issue that must be addressed before initiation of clinical trials for *in vivo* efficacy of CAP since some anti-HIV-1 agents, such as the soluble CD4,^{28,29} are much less effective in inhibiting infection of primary target cells by primary HIV-1 isolates than that by TCLA strains.

On the basis of the envelope gene sequences and coreceptor usage, primary HIV-1 isolates can be classified into different genotypes (e.g., clades A to G, and group O) and biotypes (i.e., R5, X4, and R5X4).^{30,31} Using a series of primary HIV-1 isolates with distinct genotypes and biotypes, we have demonstrated that CAP inhibits *in vitro* infection by all the tested primary HIV-1 isolates in PBMCs and MDMs (Fig. 1 and Table 1). Although using primary target cells (e.g., PBMCs and MDMs) in the virus replication assays may reflect the real conditions of HIV-1 *in vivo* infection, these assays are more time-consuming and laborious as well as may have higher experimental variation than assays using cell lines, since the sensitivity of the primary target cells isolated from different individual donors to infection by primary HIV-1 isolates is variable. Here we used CEMX174 5.25M7 cells, transduced by a luciferase reporter gene and expressing CD4 and both coreceptors CXCR4 and CCR5, for determining the inhibitory activity of CAP on infection by both R5 and X4 primary HIV-1 isolates. The results indicate that CAP inhibits infection of CEMX174 5.25M7 cells by all HIV-1 primary isolates tested, consistent with the results with PBMCs. Interestingly, the EC₅₀ values are about 3- to 20-fold lower than those obtained with PBMCs, suggesting that the assay system using CEMX174 5.25M7 cells is more sensitive in determining the inhibitory activity of compounds against primary HIV-1 isolates than that using PBMCs. The difference in sensitivity of inhibition using CEMX174 5.25M7 and PBMCs most likely reflects the difference in the duration of infection, that is, the number of rounds of replication before assessment is much smaller in CEMX174 5.25M7 cells than in PBMCs.

Vaginal or rectal infection by HIV-1 may be mediated by exposure to HIV-1-infected cells within semen through multiple mechanisms for HIV-1 transmission across the mucosal epithelium, including transcytosis of HIV-1 through epithelial cells and/or specialized M cells; epithelial transmigration of HIV-1-infected donor cells; uptake of HIV-1 by intraepithelial Langerhans cells; or circumvention of the epithelium via breaches in the epithelial integrity.^{32,33} In the last case, some epithelial damage may be required to facilitate HIV-1 transmission across mucosal epithelium and/or lead to the exposure of subepithelial dendritic cells (DCs), macrophages, and T cells, which are highly susceptible to HIV-1 infection. Experiments in the macaque model showed that SIV-infected cervical and vaginal DCs can be detected within a few hours of vaginal inoculation.³⁴ Furthermore, DCs can adsorb virions in the absence of

productive infection, as highlighted by the demonstration that HIV-1 gp120 is bound by the mannose receptor, DC-SIGN.³⁵ The migratory nature of DCs is thought to account for the rapid transport of infectious virus to draining lymph nodes after vaginal or rectal SIV inoculation of rhesus macaques.^{36,37} Here we confirmed that mature DCs are unable to replicate primary HIV-1 isolates, but are effective in transmitting HIV-1 to PBMCs. Transmission of primary HIV-1 isolates from DCs to PBMCs was inhibited by CAP in a dose-dependent manner. PBMCs that contain CD4⁺ T lymphocytes and monocytes/macrophages in blood circulation are effective in transmission of the primary HIV-1 isolates to CEMX174 5.25M7 cells and this transmission can be blocked by CAP, confirming that CAP is a potent inhibitor of HIV-1 cell-to-cell transmission.

Although some isolates appear more sensitive to CAP than others, there is no significant correlation between the sensitivity of the HIV-1 isolates to CAP and their subtypes or their coreceptor usage, suggesting that CAP-mediated anti-HIV-1 activity is independent of virus genotypes and biotypes. Furthermore, the CAP formulation that will be used in clinical trials contains CAP at about 130 mg/ml, which is more than 10,000-fold higher than the concentrations to achieve 50% inhibition of infection by most primary HIV-1 isolates tested in the present studies. These results confirm that CAP has potent anti-HIV-1 activity against a broad spectrum of cell-free and cell-associated primary HIV-1 isolates and may be further developed as a topical microbicide for worldwide application to prevent sexual transmission of HIV-1.

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FIG. 1.

Effects of CAP on cell-free and cell-associated HIV-1 infection of cervical explants. For testing cell-free virus, explants were exposed to HIV-1_{BaL} diluted in culture medium or in 25% semen in the absence or presence of CAP (1 mg/ml). After extensive washes, explants were cultured for 10 days before measuring p24 antigen. For testing cell-associated virus, explants were exposed to HIV-1_{BaL}-infected PBMCs in the absence or presence of CAP. After extensive washes, explants were cultured for 10 days, followed by measuring p24 antigen production. Data were obtained from three independent experiments using tissue from different donors and are presented as means $\pm$ SE.

FIG. 2.

CAP-mediated inhibition of infection by primary HIV-1 isolates of MDMs (A), PBMCs (B), and CEMx174 5.25 M7 cells (C). All the samples were tested in triplicate and averages are presented. The subtypes (clades) and biotypes (coreceptor usage) of the virus isolates tested are shown in each panel.

FIG. 3.

CAP-mediated inhibition of transmission of primary HIV-1 isolates from DCs to PBMCs (A), from MDMs to PBMCs (B), and from PBMCs to CEMx174 5.25M7 cells (C). All the samples were tested in triplicate and averages are presented. The subtypes (clades) and biotypes (coreceptor usage) of the virus isolates tested are shown in each panel.

Table 1

Inhibitory Activity of Cellulose Acetate 1,2-Benzenedicarboxylate on Infection of PBMCs and CEMx174 5.25M7 Cells by Primary HIV-1 Isolates

HIV-1 isolate [subtype ^a (coreceptor usage)]	EC ₅₀ (μg/ml) for inhibition of HIV-1 infection in:		
	PBMCs	MDMs	CEMx174 5.25M7
94UG103 (A, X4R5)	13.13 ± 4.76 ^b	23.49 ± 10.14	1.45 ± 0.14
92UG029 (A, X4)	94.69 ± 4.77	ND	3.56 ± 0.03
92RW008 (A, R5)	24.04 ± 5.40	113.73 ± 19.08	1.73 ± 0.47
92RW023 (A, R5)	11.01 ± 0.02	ND	ND
92BR018 (B, R5)	83.40 ± 9.26	ND	ND
92US657 (B, R5)	57.28 ± 14.24	115.38 ± 6.62	1.56 ± 0.31
93MW959 (C, R5)	21.56 ± 4.24	ND	3.89 ± 0.22
93IN101 (C, R5)	9.00 ± 0.86	5.88 ± 0.64	0.58 ± 0.09
92BR025 (C, R5)	10.14 ± 2.62	ND	ND
92UG001 (D, X4R5)	42.68 ± 3.30	ND	1.80 ± 0.09
93TH051 (E, X4R5)	35.51 ± 2.25	ND	0.71 ± 0.11
92TH009 (EA, R5)	6.02 ± 2.00	75.57 ± 4.80	0.14 ± 0.03
93BR020 (F, X4R5)	38.48 ± 13.60	119.14 ± 9.84	1.69 ± 0.28
BCF02 (group O, R5)	31.75 ± 3.15	1.51 ± 0.38	10.86 ± 1.15

Abbreviations: MDM, monocyte-derived macrophage; ND, not done; PBMC, peripheral blood mononuclear cell.

^a Subtype of the *env* gene.

^b Mean ± SD.

Table 2

Inhibitory Activity of Cellulose Acetate 1,2-Benzenedicarboxylate on *in Vitro* Transmission of Cell-Associated Primary HIV-1 Isolates

HIV-1 isolate [subtype ^a (coreceptor usage)]	EC ₅₀ (μg/ml) for inhibition of HIV-1 transmission from:		
	DCs to PBMCs	MDMs to PBMCs	PBMCs to 5.25M7
94UG103 (A, X4R5)	4.23 ± 0.06 ^b	9.88 ± 2.32	3.50 ± 0.47
92UG029 (A, X4)	22.27 ± 5.68	ND	37.61 ± 1.87
92RW008 (A, R5)	6.85 ± 1.90	4.84 ± 0.98	15.57 ± 0.73
92US657 (B, R5)	66.52 ± 9.87	6.84 ± 1.84	28.41 ± 2.65
93MW959 (C, R5)	ND	ND	10.55 ± 1.40
93IN101 (C, R5)	1.66 ± 0.04	8.02 ± 0.78	4.33 ± 0.81
93UG065 (D, X4)	ND	ND	7.69 ± 1.69
92UG001 (D, X4R5)	63.86 ± 1.07	ND	37.20 ± 0.37
93TH051 (E, X4R5)	16.97 ± 0.18	ND	2.95 ± 0.26
CMU02 (EA, X4)	50.77 ± 5.95	ND	6.90 ± 0.19
CMU06 (E, X4)	ND	ND	21.16 ± 1.69
92TH009 (EA, R5)	ND	16.14 ± 2.40	ND
RU570 (G, R5)	20.43 ± 4.51	ND	31.65 ± 3.88
BCF02 (group O, R5)	15.18 ± 2.83	83.32 ± 1.87	6.89 ± 0.69

Abbreviations: DC, dendritic cell; MDM, monocyte-derived macrophage; ND, not done; PBMC, peripheral blood mononuclear cell.

^a Subtype of the *env* gene.

^b Mean ± SD.