

Evaluation of Anti-HIV-1 Microbicide Potency of Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) UC781

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ABSTRACT

At the beginning of Acquired Immunodeficiency Syndrome (AIDS) epidemic in the mid of 1981, a few hundreds of people were identified with human immunodeficiency virus type-1(HIV-1) infection and AIDS. The infected population increased to a total 7.3 million in 1990 and within 10 years, the rapid spread of infection reached an average of 28 million people in 2000, and was recently estimated at 36 million at the end of 2015. However, highly active antiretroviral therapy (HAART) in HIV-infected patient does not show signs and symptoms of AIDS and has shown to delay death among patient. There have been no declines in new HIV infection among adults and 1.9 million adults have become newly infected with HIV since 2010. A strategy to minimize the sexual transmission of HIV is to develop a topical microbicides alternative of vaccine. UC781 is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and which has significant anti-HIV-1 microbicidal activity. One of the concerns about the use of NNRTI as a microbicide is that they fail to prevent the transmission of NNRTI-resistant virus. In this study, HIV-1 resistant to UC781 varied with EFVR> UCR>NVPR>WT. These observations suggested that UC781 needs further clinical analysis for use in topical anti-HIV microbicide.

Keywords

HIV/AIDS, microbicide, UC781, NRTI-resistant, NNRTI-resistant.

Abbreviations

AIDS: Acquired Immunodeficiency Syndrome, HIV: human immunodeficiency virus type-1(HIV-1), NNRTI: non-nucleoside reverse transcriptase inhibitor, WT: wild-type, UCR: UC781-resistant, NVPR: Nevirapine-resistant, EFVR: Efavirenz-resistant

Introduction

According to estimates by the United Nations Acquired Immunodeficiency Syndrome (UNAIDS), 36.7 million people were living with HIV in 2015 [1]. Approximately, 78 million people have become infected with HIV and 35 million people have died from AIDS-related illnesses since the epidemic began in the late 1970s and early 1980s. Recently, the U.S. Centers for Disease Control and prevention reported that there are still sharp increases in infection rates among young black men who have sex with other men [2]. Vaccine would be most useful method

to tackle the current HIV/AIDS epidemic; unfortunately, despite enormous effort, anti-HIV vaccines are not yet available. So, an alternative new method is needed to control the risk of sexual HIV acquisition. Microbicides could be used to protect against sexually transmitted infections (STIs) including HIV. There is an urgent need to investigate safe and effective topical microbicides to stop worldwide catastrophe of HIV-1 infection.

Topical anti-HIV microbicides are products designed to block or prevent the transmission of HIV when applied in the vagina/rectum or any mucosal surfaces sensitive to HIV infection [3,4]. An ideal microbicide should fulfill a number of criteria including high potency against HIV-1, the ability to directly inactivate the virus, efficacy against a wide range of HIV strains, prevention of cell-to-cell transmission of HIV, and provide a barrier to viral infection of uninfected cells. Based on mechanisms of action against HIV, a microbicide candidate should target any of the ten main steps of HIV life cycle to prevent infection: binding, fusion, reverse transcription, proviral DNA integration, host-cell activation, transcription, translation, protein assembly, budding or viral

release. A variety of compounds that inhibit HIV-1 replication are now under investigation and some of them are already in clinical trials [5]. The potency of compound in anti-HIV microbicides is categorized based on their ability to stop HIV replication: non-specific, moderately specific and highly specific [6,7]. Drugs that terminate the function of reverse transcription activity can be divided into two main classes; nucleoside (or nucleotide) reverse transcriptase inhibitors (NRTIs) and non-NRTIs (NNRTIs). NRTIs can be used as a potential agent for anti-HIV microbicides [8]; however, none have been successfully formulated as fully active anti-HIV microbicides nor available commercially. Currently, NNRTI compounds in development as microbicides include UC781, dapivirine (TMC120), MIV 150, and S-DABO (HI 280) [9-12].

UC781 is a non-nucleoside reverse transcriptase inhibitor (NNRTI); the specific mechanism of action involves binding tightly to HIV replication enzyme (reverse transcriptase) and inhibiting HIV-1 infection. UC781 has shown potent antiviral activity against wild-type HIV-1 [13,14]. In the previous studies, anti-HIV microbicide activity of UC781 was tested [15,16]; and it was proposed that it could be an important component in anti-HIV-1 topical microbicides. Recently, UC781 has been considered as a candidate for an anti-HIV microbicides that can prevent the sexual transmission of HIV under investigation in clinical trials. However, question that have been raised whether NNRTI-based microbicides is effective against NNRTI-resistant HIV-1. Possibly, NNRTI-based microbicides will select for the transmission of NNRTI-resistant virus strains. We therefore generated NNRTI-resistant HIV to evaluate the efficacy of UC781 against resistant HIV variants. A variety of microbicide-relevant tests were performed including testing of the antiviral activity of UC781 in pretreated cell to protect cells from subsequent infection in the absence of exogenous drug, inactivation of cell-free virus, inhibition of cell-to-cell HIV-1 transmission [11].

Microbicidal Properties of UC781

A microbicide should be colorless, odorless, affordable, stable, and widely acceptable. It should be stable under a variety of storage condition and last longer in vaginal or anal environments.

Characteristics of UC781	References
UC781 is a tight-binding inhibitor	16
Penetrates the HIV-1 membrane envelope and capsid core	15
Having virucidal activity after drug removal	12
High potency against wide range of HIV-1 strains	25
Inactivate cell-free and cell-associated virus	11
Microbicidal activity against resistant HIV: EFVR> UCR>NVPR>WT	11
Prevents transmission of cell-associated wild-type HIV-1	11
Long-term exposure required to inhibit NNRTI-resistant HIV-1	11
To inhibit NNRTI-resistant HIV, high conc. of UC781 needed	11
Cannot prevent cell-to-cell transmission of NNRTI-resistant HIV	11
Select transmission of NNRTI-resistant in pretreated cells	11
Should have minimum toxicity against human cells	14

Table 1: Microbicidal properties of UC781.

To be microbicides, UC781 must possess some special characteristics that fulfil its utility as an anti-HIV-1 microbicide to prevent sexual transmission HIV-1 (Table 1).

Microbicidal effect of UC781 on cell-free and cell-associated HIV

Anti-HIV-1 microbicides must function at several levels to inhibit HIV transmission. Endogenous reverse transcription has been reported to enhance virus infectivity and facilitate sexual transmission of the virus [17]. First, UC781 inactivates cell-free virus to prevent sexual transmission of HIV. Evidently, UC781 readily permeates the viral envelope and the capsid core of HIV virions and binds tightly to the viral reverse transcriptase (RT). HIV-1 infectivity is therefore reduced due to inhibition of endogenous reverse transcription in the free virion. Dramatic inhibition of endogenous reverse transcription inside the virions can occur when free virions are exposed to UC781. Our previous studies reported that short exposure of isolated wild type HIV-1 virions to nanomolar concentrations of UC781 completely inactivated the virus and thus inactivation persist long after removal of extravirion UC781 [11,15,16]. The compound should act directly on the cell-free virus and thereby prevent infection. Therefore, endogenous reverse transcription has been considered as one of the most important factor for the control of sexual transmission of HIV-1. Second, UC781 attenuates the infectivity of nascent HIV-1 produced by infected cells after exposure to UC781, thereby inhibiting transmission of cell-associated virus. Finally, UC781 is readily taken up and remains for extended periods within uninfected HIV-susceptible cells. HIV infection can be inhibited by UC781 residing in cell membrane during entry of HIV into the cells. This protective effect is established in less than 10 minutes after exposure to UC781. A vaginal microbicidal formulation to prevent sexual transmission of the virus was an important consideration for the use UC781.

Microbicidal activity against NNRTI-resistant HIV

Emergence of drug-resistant HIV is crucial dilemma for the prevention of HIV-1 transmission by microbicides. Recent studies have shown that mutant strains of HIV-1 are increasingly prevalent in infected individuals not just in developed countries but also in other developing countries in the world. In mothers, receiving single-dose nevirapine alone resulted in resistance rates ranging from 25% to 69% [18]. In our previous studies, NVPR, UCR, and EFVR virus strains were generated in the serial passage of HIV-1 in the presence of increasing concentration of UC781 (Figure 1). There were three mutations developed against UC781 and the UC781-resistant strain is highly resistant to UC781. Genital secretions of NNRTI-experienced women harbor K103N and K238N mutations in resistant strains of HIV-1 and without drug selection these variants can exist for years [19]. Several mutant strains have been characterized as resistant to NNRTI including V106A+ F227L+L100I and Y181C+V108I+K101E [20]. NNRTI-resistant strains were developed in the presence of increasing concentration of UC781, efavirenz and nevirapine under drug pressure in vitro. The microbicidal activity of UC781 against UC781-resistant (UCR, V106A+ I135R + Y181C), efavirenz-

resistant (EFVR, L100I+K103N) and nevirapine-resistant (NVPR, Y181C) strains were evaluated using a number of tests, including inhibition of cell-to-cell HIV-1 transmission and inactivation of cell-free virus. UC781 was 10 – 100-fold less effective against NNRTI-resistant HIV-1 compared to wild type (wt) virus in each of these tests. EFVR and UCR were highly and moderately resistant to UC781 respectively. Microbicidal activity of UC781 against different virus strains were reported as wt ≥ NVPR > UCR ≥ EFVR (Table 2). Due to tight-binding antiviral activity of UC781, HIV becomes resistant to UC781 very easily. UC781 is ineffective against UC781-resistant viruses even in many cases. For example, high amount of UC781 is needed to inhibit replication of UC781-resistant and other NNRTI-resistant viruses. However, high doses of UC781 can be toxic to cells and causes cell death.

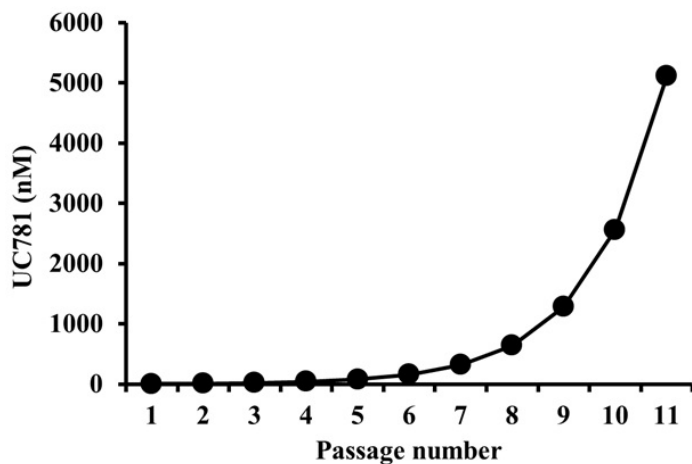


Figure 1: Development of NNRTI-resistant HIV-1 *in vitro*. UC781 resistant HIV-1 was generated by serial passage of HIV-1 NL4-3 in the presence of increasing concentrations of UC781.

Virus Strain	Subtypes (env)	Co-receptor usage	Phenotypes	Resistant to UC781	Ref
Wild-type	B	X4	SI	(-)	11,16
EFV ^R	B	X4	SI	(+++)	11
NVP ^R	B	X4	SI	(+)	11
UC ^R	B	X4	SI	(++)	11

Table 2: Microbicide activity of UC781 against UC781-resistant HIV-1. EFV^R= Efavirenz resistant; NVP^R= Nevirapine resistant; UC^R= UC781 resistant. (+ + +) = Highly resistant; (++) = Moderately resistant; (+) = Weakly resistant; (-) = Not resistant. SI= Syncytium inducing; X4= CXCR4.

Microbicidal activity of UC781 on cell-to-cell HIV-1 transmission

HIV-infected patient seminal and vaginal fluids contain both free virus particles and virus-infected cells [21] and cell-associated virus has been reported to be a more significant source for HIV transmission than cell-free virus [22,23]. The ideal microbicide must effectively block transmission of cell-free and cell-associated virus, as both may be present in genital tract fluids [23]. We evaluated the microbicidal activity of UC781 against UC781-resistant (UCR), efavirenz-resistant (EFVR) and nevirapine-resistant (NVPR) strains in a microbicide relevant test, including

inhibition of cell-to-cell HIV-1 transmission. UC781 pretreatment completely prevented cell-to-cell transmission of wt HIV-1 for up to 72 hrs following removal of UC781. In contrast, UC781 pretreatment was ineffective at preventing cell-to-cell transmission of UCR strain of HIV-1 [11]. UC781 does not carry direct virucidal property and due to drug selection the resistant virus that escapes may infect susceptible cells. The combination therapies are the only useful approach rather than alone. The microbicidal research area unfortunately has not yet efficiently considered this fact. Thus, combining UC781 with other component that acts at different steps in the HIV life cycle will be potentially advantageous. Components to consider in combination with UC781 might be any type of HIV-1 entry inhibitor. Therefore, a potential combination would be to combine UC781 with an antiretroviral agent which can prevent transmission of resistant virus.

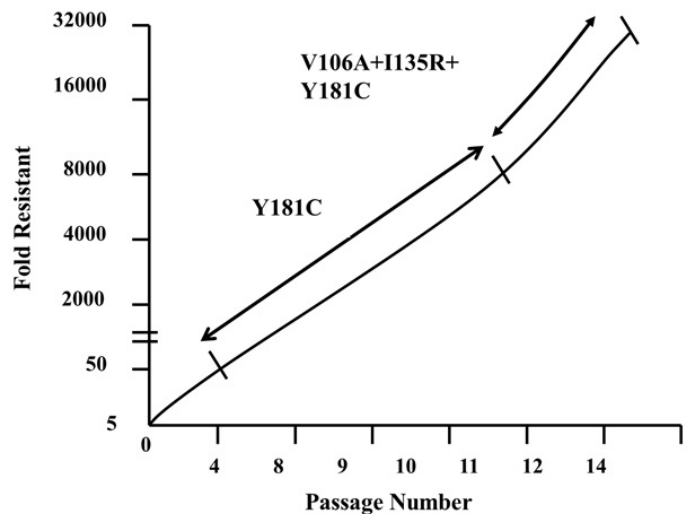


Figure 2: Generation of UC781-resistant mutants. Wild-type HIV-1-infected MT2 cells were passaged in the presence of increasing 2-fold serial dilution of UC781. Mutations were detected in HIV-1 proviral DNA after sequencing.

Evaluation of Broad spectrum microbicidal activity of UC781

The preclinical evaluation of antiviral activity using different cells types is an essential part of the developmental process for a potential microbicide. Since, the actual process by which HIV is transmitted through sexual contact has not been fully elucidated, it is necessary to test candidate compounds for antiviral activity using a variety of cell and virus combinations [24]. Focusing on cell types known to be targets for virus infection *in vivo* and the virus types believed to be the most likely to transmit infection. In these studies, cells were pretreated with UC781 whether cells can prevent spread of virus from cell-to-cell and virus becomes inactivated during entry into the cells. Twenty-five different HIV-1 strains were tested to evaluate anti-HIV microbicide activity of UC781 in single cycle HIV-1 infection assay, activated human peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs) [25]. Notably, UC781 blocks infection of PBMCs that use either CCR5 or CXCR4 co-receptors by HIV-1 strains belonging to clades A, B, C, D, E, F, G, O, and N. All the clades are distributed throughout Africa, Asia, Europe,

and America. UC781 was found to be active against X4, R5, and dual-tropic HIV-1 strains in CD4+ T cells, PBMCs, macrophages, and double co-receptor cell line, suggested that UC781 might have broad-spectrum anti-HIV-1 microbicide activity in vitro.

Conclusion

UC781 has been found to have excellent anti-HIV microbicide activity while fulfilling all the criteria to formulate an anti-HIV microbicide. Characterization of HIV-1 resistant to UC781 is one of the main objectives of preclinical evaluation of candidate microbicide. The higher concentrations of UC781 were required to inactivate NNRTI-resistant HIV-1, especially UCR and EFVR virus, suggesting that UC781 have but not in full microbicidal activity against HIV-1 strains with either the V106A+I135R+Y181C or the L100I+K103N mutations in RT. However, prevention of HIV-1 transmission by UC781 from different geographical region may not be a significant issue at current microbicide formulation. The reduced microbicidal effect of UC781 against NNRTI-resistant HIV-1 suggests that NNRTI-based microbicide formulations might best be developed as combination microbicides containing one or more additional antiviral agents. The finding in this study suggests that full protection from HIV-1 infection using NNRTI-based microbicides is still out of reach [11,26,27].

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