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Inhibition of endogenous reverse transcription of human and nonhuman primate lentiviruses: Potential for development of lentivirucides

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Abstract

In the current study, we extended our previous works on natural endogenous reverse transcription (NERT) and further examined its potential as a virucide molecular target in sexual transmission of primate lentiviruses. HIV-1 and SIV virions were pretreated with select nucleoside (NRTIs) and nonnucleoside RT inhibitors (NNRTIs), either alone or in combination with NERT-stimulating substances. The effects of these antiretrovirals on virion inactivation were analyzed in human T cell lines and primary cell cultures. Pretreatment of HIV-1 virions with physiologic NERT-stimulants and 3′-azido-3′-deoxythymidine 5′-triphosphate (AZT-TP) or nevirapine potently inactivated cell-free HIV-1 virions and resulted in strong inhibition of the viral infectivity. Pretreatment of chimeric SHIV-RT virions with NERT-stimulating cocktail and select antiretrovirals also resulted in virion inactivation and inhibition of viral infectivity in T cell lines. Our findings demonstrate the potential clinical utility of approaches based on inhibiting NERT in sexual transmission of HIV-1, through the development of effective anti-HIV-1 microbicides, such as NRTIs and NNRTIs.

Keywords: HIV-1; SIV; NERT; NRTI; NNRTI; RT; Virucide

Introduction

Reverse transcriptase (RT) represents a virally encoded protein with three enzymatic activities, RNA- and DNA-dependent DNA polymerase and RNase H (Baltimore, 1970; Coffin, 1990; Temin and Mizutani, 1970). Previous studies have shown that these activities can be measured either within virions made permeable by low levels of detergent or amphipathic peptides, such as melittin, to allow access of deoxyribonucleoside triphosphates (dNTPs) (Boone and Skalka, 1981a, 1981b; Garapin et al., 1970; Gilboa et al., 1979) or by complete disruption of the virions with high concentrations of nonionic detergents (Poiesz et al., 1980), representing endogenous and exogenous RT activity, respectively. Endogenous RT (ERT) reactions have been a key in the evaluation of the kinetics and molecular intermediates of reverse transcription (Boone and Skalka, 1981a, 1981b; Borroto-Esoda and Boone, 1991; Gilboa et al., 1979). Early studies have demonstrated that nonionic detergents are helpful in permeabilizing retroviral virions to allow near full-length viral DNA synthesis (Gilboa, 1979; Yong et al., 1990). Nevertheless, endogenous RT activity was also noted in nonpermeabilized HIV-1 virions, by several groups, including ours (Yong et al., 1990; Borroto-Esoda and Boone, 1991; Debyser et al., 1992; Zhang et al., 1993; Zhang et al., 1996b, 1996c). We and others have demonstrated that in a detergent-free system, addition of dNTPs to isolated HIV-1 virions could stimulate RT activity, leading to higher levels of virions carrying strong stop negative strand moieties and more complete negative strand intermediates (Borroto-Esoda and Boone, 1991; Zhang et al., 1996b). The intravirion reverse

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transcripts could be detected in HIV-1 virions, not just in vitro but also in vivo (Zhang et al., 1994, 1996a). The efficiency of intravirion reverse transcription in HIV-1 virions is augmented by certain physiologic substances such as polyamines (e.g., spermine and spermidine). It is notable that polyamines reach very high concentrations in seminal fluids. HIV-1 virions in seminal plasma were found to harbor dramatically higher levels of full-length or nearly full-length reverse transcripts, as compared with virions isolated from the peripheral blood of HIV-1-seropositive men (Zhang et al., 1996b). HIV-1 virions, which have processed a certain amount of ERT, would have a kinetic advantage to infect initially resting CD4 T cells and macrophages (Zhang et al., 1996b; Dornadula et al., 1999). As intravirion reverse transcription is a biochemically active process that can take place without nonphysiological virion permeabilization, it has been entitled "natural endogenous reverse transcription (NERT)" (Zhang et al., 1996b, 1998).

We have further demonstrated that the amphipathic domains at the C-terminus of HIV-1 gp41 are able to make the viral envelope naturally permeable to dNTPs, which underlines the molecular mechanism of NERT phenomenon (Zhang et al., 1996c). Deletion of these amphipathic domains can eliminate the exogenous dNTP-driven intravirion reverse transcription (Zhang et al., 1996c). It has been demonstrated that the peptides derived from the amphipathic domains at the C-terminus of gp41 [named as lentiviral lytic peptide (LLP)] are able to lyse either prokaryotic or eukaryotic cells (Miller et al., 1991; Miller et al., 1993). These peptides can also form a pore in planar phospholipid bilayer membrane (Chernomordik et al., 1994). We have found that the synthesized LLP, like melittin, can allow dNTPs to pass through viral envelope which lacks the LLP domain (Zhang et al., 1996c).

At this stage in AIDS pandemic, prevention of sexual transmission of HIV-1 is of highest priority. Many investigators have attempted to develop various virucides to inactivate the cell-free virions and therefore prevent the sexual transmission of HIV-1 (Balzarini et al., 1998; Fauci, 1993; Fichorova et al., 2005; van Damme and Rosenberg, 1999). As the viral envelope is naturally permeable to triphosphates of nucleosides and polyamines in human seminal plasma can significantly enhance the NERT activity such that a large amount of reverse transcripts can be found within cell-free HIV-1 virions in seminal fluids, we propose that intravirion reverse transcription is an ideal target for inactivating the virions in seminal fluids. We have now extended our previous studies (Dornadula et al., 1997, 1999; Zhang et al., 1993, 1994, 1996a, 1996b, 1996c, 1998) and herein present recent findings on inhibiting NERT by means of select agents and its potential usage to prevent sexual transmission of human and nonhuman primate lentiviruses.

Results

As HIV-1 virions are naturally permeable to triphosphates of nucleosides (e.g., dNTPs), we hypothesized that triphosphates of chain terminators could also pass through the viral envelope. We first conducted experiments to test the effect of the NRTI [e.g., 3′-azido-3′-deoxythymidine 5′-triphosphate (AZT-TP)] or NNRTI (e.g., nevirapine) on inhibiting NERT. As shown in Fig. 1, pretreatment of virions (200 ng of HIV-1 p24 equivalent) with 4 μM of the NNRTI, nevirapine, led to decreased NERT, as compared to NERT-stimulated controls (virions pretreated with polyamines and dNTPs at physiological concentration in seminal fluids) (Zhang et al., 1996b) by analysis of intravirion DNA, but somewhat less than AZT-TP (approximately 90% versus 95% decrease in *gag* copy numbers by phosphor-imager analyses). As expected, pretreatment of virions with AZT, which was used as a negative control in our experiments, did not affect NERT.

We have also analyzed the effects of nevirapine and AZT-TP on inactivating virions in initially quiescent human PBL cultures, which were infected with antiretroviral agent (nevirapine, AZT-TP and/or AZT at 4 μM) pretreated or untreated virions. HIV-1_{NL4-3} (X4-tropic) virions were pretreated with different concentrations of antiviral reagents, with or without NERT-stimulating cocktail at physiological concentrations in seminal fluids and then used to infect initially quiescent human peripheral blood lymphocytes (PBLs). Both nevirapine and AZT-TP demonstrated a potent inhibitory effect (approximately a 25-fold decrease) on viral infectious titer (Fig. 2a), indicating that these NERT inhibitors indeed function as virucides and effectively inactivate the virions. We have also tested various concentrations of NERT inhibitors upon viral infectivity. As shown in Fig. 2b, both AZT-TP and nevirapine again exerted a dramatic inhibitory effect on viral growth in PBL cultures 12 days post-infection, but only in the presence of the NERT-stimulating cocktail. AZT-TP, at a concentration of 1 μM and in the presence of polyamines/dNTPs, was found to be a more potent inhibitor of NERT, as compared to nevirapine at the same

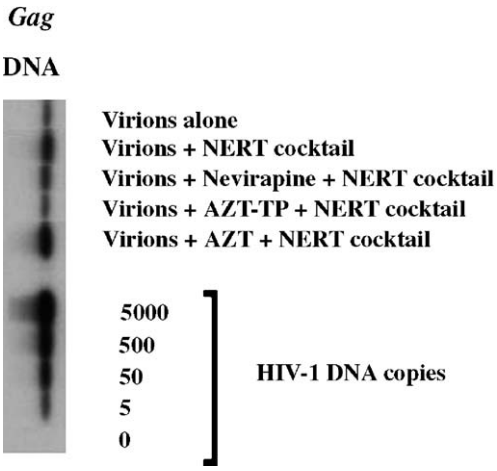

Fig. 1. Inhibitory effects of select antiretroviral agents on NERT. Intravirion HIV-1 reverse transcripts were assayed by DNA-PCR. Virions were pretreated with nevirapine, AZT-TP and/or AZT at 4 μM, with or without NERT-stimulating cocktail. After 4 h, the DNA was extracted and subjected to semi-quantitative PCR and Southern blotting analysis. The figure is representative of two independent studies.