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EVALUATION OF NEVIRAPINE PERMEABILITY AND RETENTION IN HIV-1 CELL-FREE AND BUDDED VIRIONS

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Introduction

The HIV/AIDS pandemic is continuously spreading at a rate of over 15,000 new infections every day. Heterosexual transmission is the leading mode of HIV-1 infection worldwide, with women particularly vulnerable to HIV infection. In the absence of an effective prophylactic anti-HIV therapy or vaccine, current efforts are aimed to developing retrovirucides or topical microbicides formulations of anti-HIV agents. Microbicides would provide protection by directly inactivating HIV. A safe and efficacious anti-HIV microbicide is not yet available despite the fact that more than 60 candidate agents have already been identified to have in vitro activity against HIV, 18 of which have advanced to clinical testing. Potential retrovirucides or vaginal microbicides include non-nucleoside reverse transcriptase inhibitors (NNTRI). Since their discovery, NNRTIs have become one of the cornerstones of highly active anti-retroviral therapy (HAART). Currently, three NNRTI agents, efavirenz, nevirapine and delavirdine are commercially available. A microbicide needs to be active against both free and cell-associated virus. Ideally, a retrovirucidal agent should act directly on the virus and/or to be incorporated into nascent virion during assembly and budding. Here we evaluated the permeability of nevirapine NNTRI inhibitor (NVP) and its incorporation on nascent virus particles. We developed a new strategy for directly testing the effect of potential microbicide agents on virus preparation based on Real Time PCR detection which correlated well with classical infectivity assays.

Methods

MT4 cells were infected by spinoculation using NL4-3 virus (M.O.I. 0.01). The infection was monitored by microscopic assessment until the start of cytopathic effects (syncytium formation). Infected cells were washed to remove viral particles still attached to the cells, and seed in 12 wells plate in the presence of increasing concentrations of nevirapine. Viruses released in the presence of nevirapine were recovery after over-night exposition to NVP. The residual NVP in this supernatant was removed by dialysis using molecular porous membrane against RPMI medium. With this treatment the unique NVP remaining in the preparation is the drug associated to RT inside viral particles, which was incorporated during virus budding. To test for NVP effect on virus produced from treated cells, aliquots of dialyzed and non-dialyzed supernatants were tested by Natural Endogenous Reverse Transcription Reaction (NERT) and HeLa Magi cells infectivity. NERT reaction is based on reverse transcription of a 181bp fragment corresponding the strand (-) strong stop DNA (ssDNA) in intact virions exposed to dNTPs. We developed a Real time PCR-based assay to measure the absolute quantification of NERT activity. HeLa Magi cell is an indicator cell line that express the β-gal gene under the control of the HIV LTR promoter. We further compared NERT reactions and infectivity of the nascent virus prior and after dialysis.

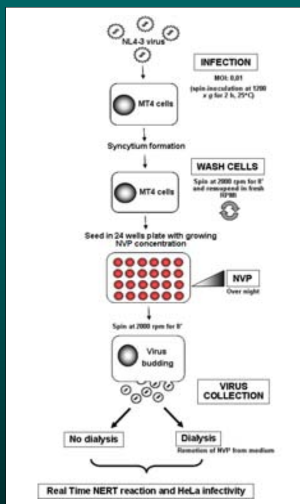

Figure 1: overview of experiments

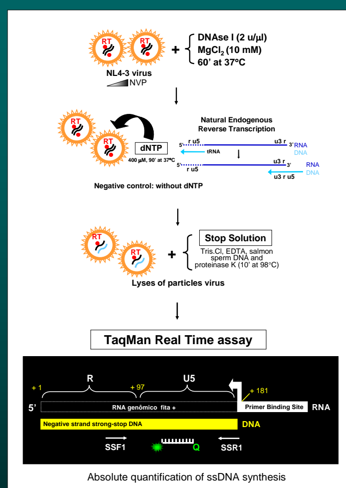

Figure 2: NERT scheme

Results

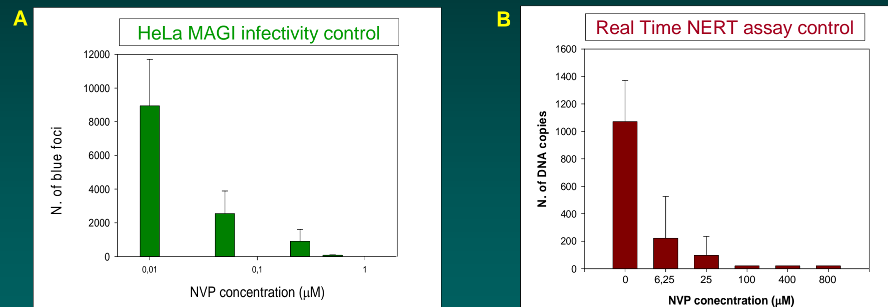

Figure 1

Control experiments: The susceptibility of NL4-3 cell-free viruses to NVP growing concentration was evaluated by HeLa MAGI infection (A) and Real Time NERT assay (B).

Conclusions

- NVP inhibits NL4-3 infectivity in HeLa cells with an IC50 value of 0.05μM while cell free viruses are inhibited in NERT assays with an IC50 of 6,0μM.
- NERT reactions associated with Real Time PCR detection is an efficient assay for testing candidate microbicide agents since it correlates well with classical infectivity tests.
- IC50 for cell free viruses was demonstrated to be 100-fold higher than for HeLa MAGI cell infections, suggesting that cell-free viruses are less permeable to NVP.

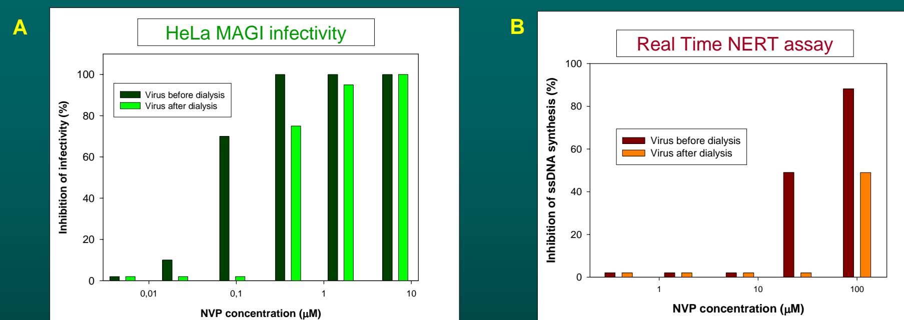

Figure 2

Inhibition of HeLa MAGI infectivity (A) and Real Time NERT PCR (B) of cell free virus budded exposed to NVP growing concentration following of dialysis treatment to remove the residual NVP.

Conclusions

- Inhibition of RT activity by NVP was less pronounced in viruses dialyzed after harvesting from cells exposed to NVP when compared with non-dialyzed counterparts. We conclude that since NVP does not tightly bind to RT, it can be excluded from viruses during budding and/or diluted out during dialysis.

General Conclusions

These data suggest that NVP penetrates the HIV-1 membrane envelope and capsid core readily inactivating the virus. However, the characteristics of NVP inhibition such as lower permeability in cell free virus and exclusion of nascent virus during the budding reduces the efficacy of NVP in microbicides strategies.

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