Degradation of SAMHD1 specifically decreases the efficacy of thymidine HIV RT analog inhibitors

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ABSTRACT

BACKGROUND: SAMHD1 has recently been recognized as an antiviral factor that acts by depleting deoxynucleoside triphosphates (dNTPs) availability for HIV reverse transcriptase (RT), via hydrolization of dNTPs. SAMHD1 restriction is counteracted by the HIV-2 accessory protein Vpx, which targets SAMHD1 for proteosomal degradation, resulting in an increased availability of dNTPs and consequently enhanced viral replication. Nucleoside reverse transcriptase inhibitors (NRTIs) are the most commonly agents used in antiretroviral therapy. NRTIs inside the cell compete with cellular dNTPs as substrate for viral RT. Consequently, SAMHD1 activity has been reported to influence NRTI efficacy in inhibiting viral replication. Here, a panel of different RT inhibitors were analysed in views of their different antiviral efficacy depending on SAMHD1.

METHODOLOGY: Antiviral activities of different NRTIs (AZT, ddC, 3TC, d4T and TDF), NNRTIs (NVP, EFV) and integrase inhibitor Raltegravir were assessed in primary cells expressing SAMHD1 (MDM and PBMCs) or the MT-4 cell line. Cells were infected either with a VSV-pseudotyped HIV-1 GFP-expressing virus or alternatively with GFP-expressing HIV-2 virus with or without Vpx. For HIV-1 infections, Vpx expression was achieved by cell transduction with viral like particles containing Vpx (VLP-Vpx). Replication levels were measured by flow cytometry and 50% effective concentrations (EC_{50}) were determined.

RESULTS: Modulation of SAMHD1 cellular levels either with VLP-Vpx or by HIV-2 infection resulted in a significant reduction of SAMHD1 protein expression in MDM and PBMCs that was accompanied of an increase in viral infection (5 and 2 fold increase for MDM and PBMCs, respectively). Contrary, no effect was observed in the MT-4 cell line where SAMHD1 expression was not detectable. EC50 where obtained for all the inhibitors in MDMs and MT-4 cells infected with HIV-1 with or without VLP-Vpx. As expected, no changes in sensitivity to NNRTIs or integrase inhibitors where observed in MDMs with or without VLP-Vpx. In the case of NRTIs, sensitivity significantly changed in the case of the thymidine analogs (10 and 9 fold difference in EC50 for AZT and d4T, respectively), but no differences were observed with 3TC, ddC and TDF. Accordingly, sensitivity to AZT was also reduced in PBMCs infected with HIV-2 compared to infection with HIV-2 ΔVpx strain, although to a lesser extent. No changes in sensitivity to any of the inhibitors were observed in MT-4 cells in the presence or absence of Vpx.

CONCLUSIONS: Reduction of SAMHD1 levels significantly decreases HIV sensitivity to thymidine but not other nucleotide RT analog inhibitors in both macrophages and lymphocytes. Our results may suggest a differential contribution of the different dNTPs in HIV replication, being thymidine the most limiting nucleotide.

ABSENCE OF SAMHD1 AFFECTS AZT ANTIVIRAL ACTIVITY WITHOUT APPARENT RESTRICTION OF VIRAL REPLICATION

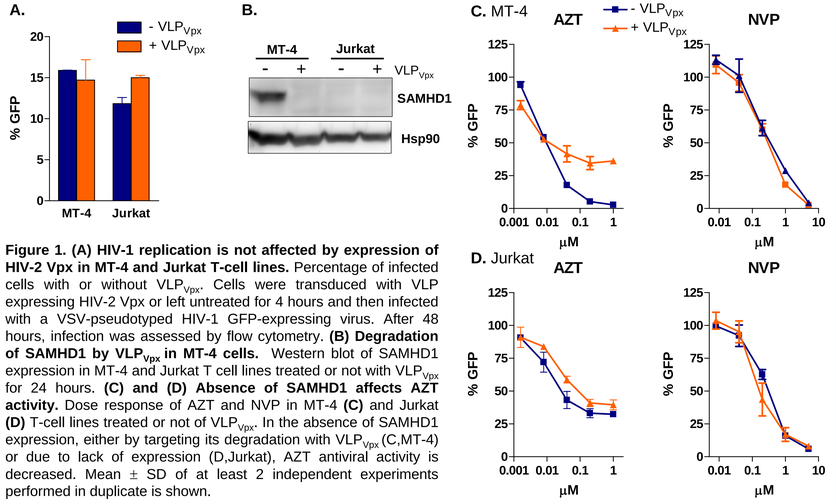


Figure 1. (A) HIV-1 replication is not affected by expression of HIV-2 Vpx in MT-4 and Jurkat T-cell lines. Percentage of infected cells with or without VLP_{Vpx}. Cells were transduced with VLP expressing HIV-2 Vpx or left untreated for 4 hours and then infected with a VSV-pseudotyped HIV-1 GFP-expressing virus. After 48 hours, infection was assessed by flow cytometry. **(B) Degradation of SAMHD1 by VLP_{Vpx} in MT-4 cells.** Western blot of SAMHD1 expression in MT-4 and Jurkat T cell lines treated or not with VLP_{Vpx} for 24 hours. **(C) and (D) Absence of SAMHD1 affects AZT activity.** Dose response of AZT and NVP in MT-4 **(C)** and Jurkat **(D)** T-cell lines treated or not of VLP_{Vpx}. In the absence of SAMHD1 expression, either by targeting its degradation with VLP_{Vpx} (C,MT-4) or due to lack of expression (D,Jurkat), AZT antiviral activity is decreased. Mean ± SD of at least 2 independent experiments performed in duplicate is shown.

DEGRADATION OF SAMHD1 DECREASES EFFICACY OF THYMIDINE-ANALOG RT INHIBITORS IN MDM

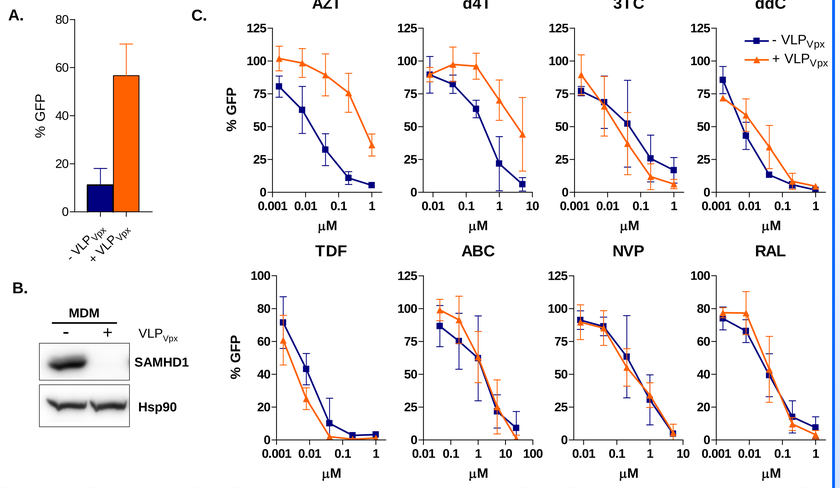


Figure 2. (A) Degradation of SAMHD1 by Vpx enhances HIV-1 replication in MDM. MDM previously treated or not with VLP_{Vpx} were infected with a VSV-pseudotyped HIV-1 GFP virus and replication assessed 2 days later by measuring GFP expression. **(B) Degradation of SAMHD1 by VLP_{Vpx} in MDM.** Western blot of SAMHD1 expression in MDM treated or not with VLP_{Vpx} for 24 hours. **(C) Decreased sensitivity of Thymidine NRTI in MDM negative for SAMHD1.** Dose response of NRTIs, NNRTIs and integrase inhibitor Ral as control. Inhibition of HIV infection was measured as the percentage of GFP+ cells relative to the no drug condition. Mean ±SD of at least three independent donors performed in duplicate is shown.

EXOGENOUS ADDITION OF dNs MIMICKS SAMHD1 DEGRADATION IN MDM

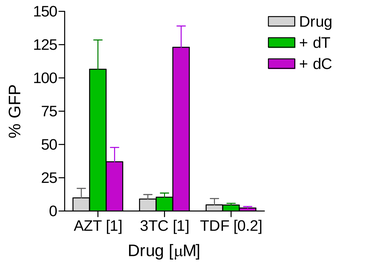


Figure 3. NRTI antiviral activity is inhibited by exogenous addition of the corresponding dN in MDM. dT or dC (1 mM) were added to the culture media together with the different NRTI 4 hours prior to infection with VSV-pseudotyped HIV-1 GFP virus and replication was assessed 2 days later. Antiviral effect of AZT or 3TC was completely lost in the presence of the corresponding dN. Mean ±SD of two independent donors performed in duplicate is shown. dT deoxythymidine; dC deoxicytidine.

DEGRADATION OF SAMHD1 INCREASES MAINLY dTTP INTRACELULAR LEVELS

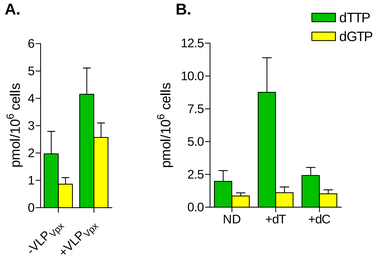


Figure 4. Intracellular dNTP levels in MDM. (A) MDM were treated or not with VLP_{Vpx} for 24 hours and intracellular dNTPs were extracted and dNTP content was determined using a polymerase-based method. dTTP was the most abundant dNTP after treatment. **(B)** dT or dC were added to the culture media for 24 hours before dNTPs were extracted and measured. As in (A) dTTP intracellular levels were increased. Mean ±SD of three independent donors is shown.

EFFICACY OF THYMIDINE NRTI IS DECREASED IN LYMPHOCYTES INFECTED WITH Vpx-CARRYING HIV

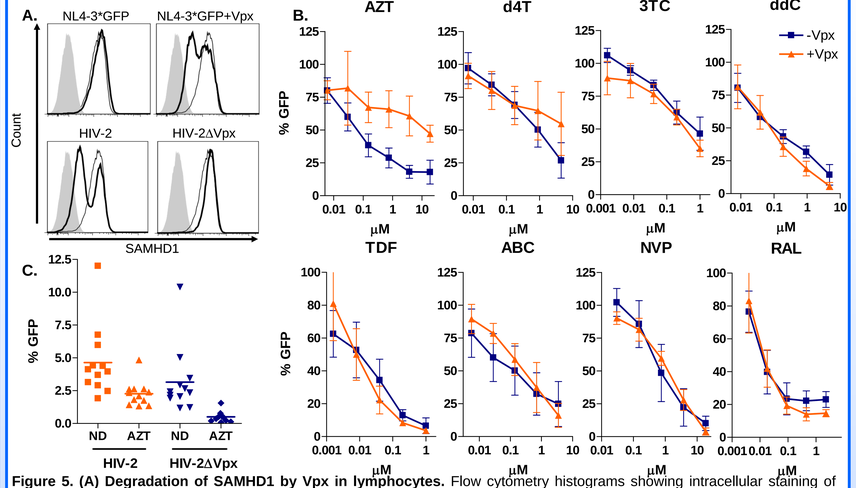


Figure 5. (A) Degradation of SAMHD1 by Vpx in lymphocytes. Flow cytometry histograms showing intracellular staining of SAMHD1 in lymphocytes infected with an NL4-3 modified to incorporate Vpx or HIV-2. **(B) Decreased sensitivity of thymidine NRTI in CD4+ T lymphocytes infected with NL4-3-Vpx virus.** Dose response of NRTIs, NNRTIs and integrase inhibitor Ral. Inhibition of HIV infection was measured as percentage of GFP+ cells relative to the no drug condition. Mean ±SD of at least three independent donors is shown. **(C) AZT antiviral potency is decreased in HIV-2 infected PBMCs *in vitro*.** PBMCs from donors were infected with GFP-expressing HIV-2 with or without Vpx and AZT (1 μg/ml) activity was assessed. ND; no drug.

SAMHD1 DEGRADATION DECREASES ANTIVIRAL POTENCY OF THYMIDINE ANALOGS IN PRIMARY CELLS

Table 1. Anti-HIV-1 activity of NRTIs (AZT, d4T, 3TC, ddC, TDF, ddI and ABC), NNRTIs (NVP and EFV) or integrase inhibitor RAL. EC50 were calculated in T-cell lines negative or expressing low levels of SAMHD1 (Jurkat and MT4)and primary cells MDM and CD4+ Tcells, both expressing SAMHD1.

		Cell type									
		Jurkat			MT-4			MDM		CD4+ T cells	
		EC_{50} (μM)		FC	EC_{50} (μM)		FC	EC_{50} (μM)	FC	EC_{50} (μM)	FC
		-Vpx	+Vpx		-Vpx	+Vpx		-Vpx	+Vpx		-Vpx	+Vpx	
DRUG	AZT	0,023	0,034	2	0,017	0,082	5	0,014	0,359	25	0,068	4,607	67
	d4T	5,000	4,300	1	0,962	2,376	2	0,300	2,009	7	1,245	0,991	1
	3TC	0,164	0,156	1	0,816	0,758	1	0,081	0,022	0	0,524	0,372	1
	ddC	0,052	0,059	1	0,292	0,308	1	0,113	0,042	0	0,115	0,081	1
	TDF	0,053	0,059	1	0,001	0,001	1	0,003	0,003	1	0,015	0,009	1
	ddI	11,598	10,858	1	2,877	2,162	1	3,175	6,333	2	1,949	6,822	3
	ABC	2,045	2,572	1	1,470	2,692	2	1,805	2,251	1	0,402	0,481	1
	NVP	0,090	0,095	1	0,237	0,295	1	0,274	0,361	1	1,017	1,256	1
	EFV	0,038	0,072	2	0,047	0,055	1	0,071	0,071	1	0,068	0,091	1
	RAL	0,003	0,007	3	0,005	0,004	1	0,022	0,031	1	0,017	0,015	1

Mean of at least three independent experiments is shown. EC50, 50% effective concentration; FC, fold change +Vpx/-Vpx.

DISCUSSION

- Downregulation of SAMHD1 expression or exogenous addition of dT significantly decreases sensitivity to thymidine NRTI and lead to an increase in dTTP intracellular pool. This suggest that competition with cellular dNTPs as substrate for viral RT might be the mechanism underlying the loss of thymidine NRTI antiviral potency and SAMHD1-mediated viral restriction.
- Decreased sensitivity to NRTI may have important in vivo implications for developing resistance to therapy, specially for HIV-2 infections.
- SAMHD1 activity is maintained in conditions where there is no apparent viral restriction.