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SAMHD1 Has Differential Impact on the Efficacies of HIV Nucleoside Reverse Transcriptase Inhibitors

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Sterile alpha motif- and histidine/aspartic acid domain-containing protein 1 (SAMHD1) limits HIV-1 replication by hydrolyzing deoxynucleoside triphosphates (dNTPs) necessary for reverse transcription. Nucleoside reverse transcriptase inhibitors (NRTIs) are components of anti-HIV therapies. We report here that SAMHD1 cleaves NRTI triphosphates (TPs) at significantly lower rates than dNTPs and that SAMHD1 depletion from monocytic cells affects the susceptibility of HIV-1 infections to NRTIs in complex ways that depend not only on the relative changes in dNTP and NRTI-TP concentrations but also on the NRTI activation pathways.

Human immunodeficiency virus type 1 (HIV-1) replicates primarily in activated $CD4^+$ T cells, while showing poor reproductive capacity in monocytes, macrophages, dendritic cells, and resting $CD4^+$ T cells (1–10). Sterile alpha motif- and histidine/aspartic acid domain-containing protein 1 (SAMHD1) is responsible for blocking HIV-1 replication in such cells (5, 11–13), reportedly by acting as a dGTP-stimulated deoxynucleotide triphosphohydrolase that hydrolyzes deoxynucleoside triphosphates (dNTPs), thus decreasing the amounts of dNTPs available for reverse transcription (3, 4, 14–19).

Nucleoside reverse transcriptase inhibitors (NRTIs) are nucleoside analogs and key components of antiretroviral therapies (20–26). They generally lack a 3′-OH group and thus act as chain terminators upon incorporation into viral DNA by reverse transcriptase (RT) (26–29). However, 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EFdA) retains a 3′-OH group, acts primarily by blocking RT translocation following incorporation of EFdA monophosphate (MP) into the template-primer, and has picomolar antiviral potency (30–37). NRTIs are administered as nucleosides and are phosphorylated to their active forms by cellular kinases (38). Hence, they compete with dNTPs for activation by cellular kinases, and their incorporation by RT is influenced by the cellular concentrations of dNTPs, which compete with NRTI triphosphates (TPs) at the RT active site (39, 40).

Amie et al. (19) recently reported that SAMHD1 does not significantly hydrolyze dideoxynucleoside triphosphates (ddNTPs) or zidovudine (AZT)-TP and that depletion of SAMHD1 in monocytic THP-1 cells decreased the potency of these NRTIs in a pseudotype-based assay. Strong evidence that the decreased potency of these NRTIs was due to increased amounts of competing dNTPs was presented. Our parallel independent study confirmed their data, extended the number of NRTIs studied, validated the results with fully infectious HIV-1, and found an unexpected disparity in the effects of SAMHD1 on the deoxyribosylthymine (dT) analogs AZT and stavudine (d4T). We demonstrate that this is due to differences in the activation of AZT and d4T, highlighting the importance of distinct metabolic pathways in NRTI activation, in addition to competition with dNTPs.

We tested purified *Escherichia coli*-produced recombinant SAMHD1 for dGTP-regulated NRTI-TP hydrolysis (using dNTPs as a reference) and separated the reaction products by anion-exchange high-performance liquid chromatography (HPLC) (14). A representative chromatogram for dATP hydrolysis by SAMHD1 is shown in Fig. 1A. Notably, NRTI-TP hydrolysis was significantly slower than that of dNTPs, with little hydrolysis after hours of incubation, rather than the minutes required for complete dNTP hydrolysis (Fig. 1B and C). The observed activity was not due to a contaminating phosphatase, as hydrolase activity was ablated by mutating the SAMHD1 active-site residue Asp207 to alanine (Fig. 1D) (14, 18, 41).

Next, we assessed whether SAMHD1 affected NRTI potency in the context of HIV-1 infection. We infected parental THP-1 cells and THP-1 cells stably expressing a SAMHD1-targeting short hairpin RNA (shRNA) (THP-$1_{KD\text{-}SAMHD1}$ cells) (42) with infectious HIV-1 carrying a luciferase reporter, in the presence or absence of the previously tested AZT and tenofovir disoproxil fumarate (TDF) (19), as well as d4T, lamivudine (3TC), dideoxyinosine (ddI), and EFdA. The knockdown efficiency of SAMHD1 in the THP-$1_{KD\text{-}SAMHD1}$ cells was >50-fold (see Fig. S1 in the supplemental material) (42). Table 1 lists NRTI 50% effective concentrations (EC_{50}s) determined in four independent experiments. As reported previously, the EC_{50}s of AZT and TDF (dT and deoxyribosyladenine [dA] analogs, respectively) were significantly increased (23- and 18-fold, respectively) in THP-$1_{KD\text{-}SAMHD1}$ cells. Notably, we observed smaller or no increases for other dT, dA, and deoxyribosylcytosine [dC] analogs (~3.5-fold increase for d4T,

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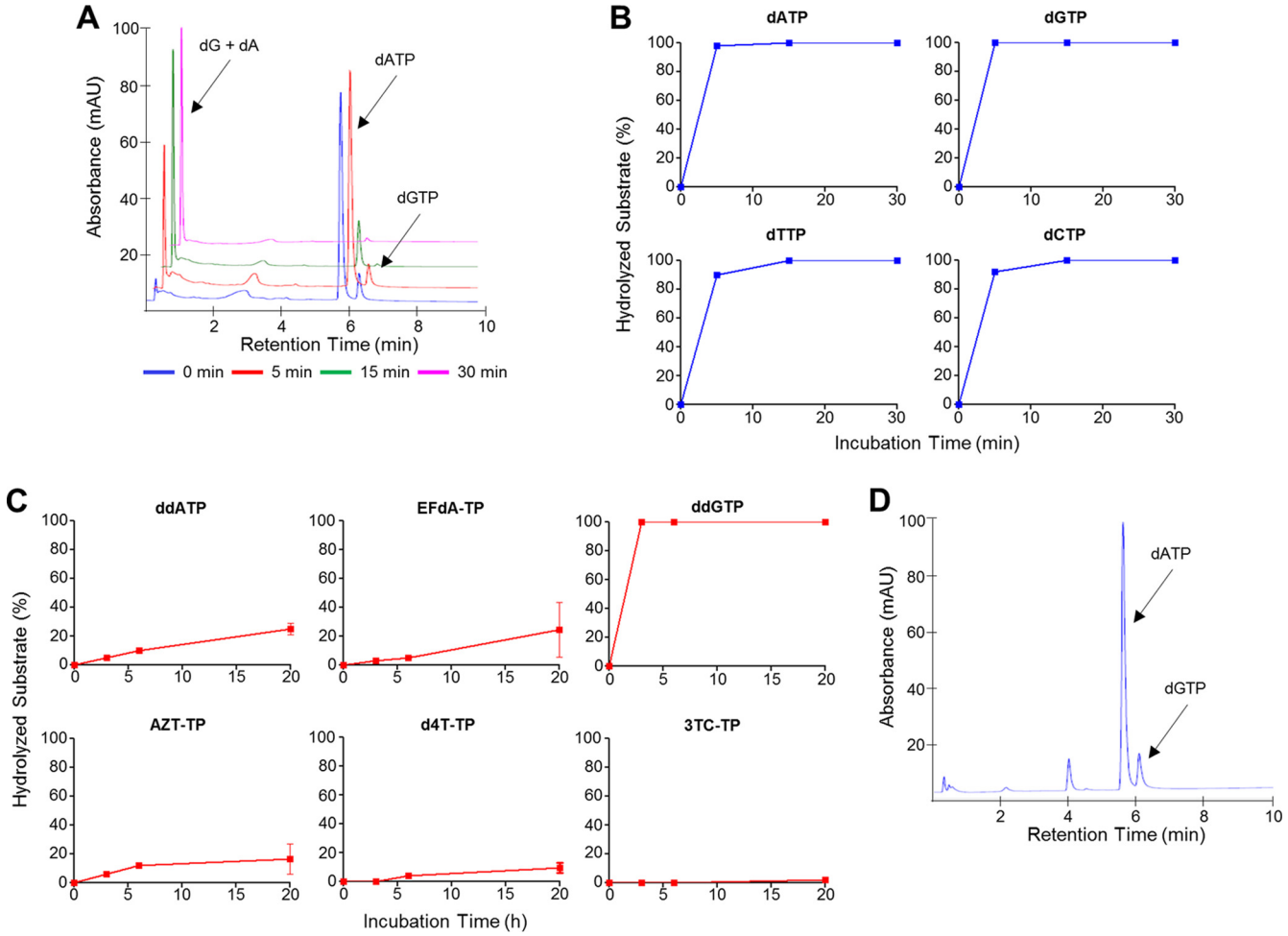


FIG 1 SAMHD1 does not efficiently hydrolyze NRTI-TPs. SAMHD1 (5 μM) was incubated at 37°C with NRTI-TP or dNTP (500 μM), in the presence of dGTP (100 μM) and $MgCl_2$ (10 mM). Reactions proceeded for 3, 6, or 20 h for NRTI-TPs and for 5, 15, or 30 min for dNTPs. Reactions were terminated by 10-fold dilution into 25 mM Tris (pH 8.0)-12.5% acetonitrile, and mixtures were analyzed by anion-exchange HPLC (DNAPac PA100 column). (A) Representative chromatograms for dATP hydrolysis. dG, deoxyribosylguanine. (B and C) Data from at least duplicate experiments for dNTP (B) or NRTI-TP (C) hydrolysis, plotted as percent hydrolysis over time (with GraphPad Prism 5). ddATP, dideoxyadenosine triphosphate; ddGTP, dideoxyguanosine triphosphate. (D) Chromatogram for dATP hydrolysis after 30 min of incubation with the SAMHD1 hydrolase active-site D207A mutant, dGTP, and $MgCl_2$.

3-fold increase for EFdA, and no increases for 3TC and ddI) (Table 1). The unexpectedly decreased potency of AZT versus d4T in THP-$1_{KD\text{-}SAMHD1}$ cells was not due to a higher rate of hydrolysis of d4T-TP by SAMHD1 (Fig. 1C) (19). It also was not caused by differences in dTTP competition with AZT-TP and d4T-TP at the RT active site, as RT incorporates AZT-TP and d4T-TP with similar efficiencies (43). Thus, we hypothesized that the different effects of SAMHD1 on the potencies of AZT and d4T involved differences in the activation pathways of the two inhibitors.

TABLE 1 EC_{50} values for NRTIs in parental THP-1 cells versus THP-$1_{KD\text{-}SAMHD1}$ cells

Drug	EC_{50} (nM) for[a]: Parental THP-1 cells	THP-$1_{KD\text{-}SAMHD1}$ cells	Fold increase in EC_{50}
AZT	1.1 ± 0.5	25.3 ± 14.9	23
TDF	0.08 ± 0.01	1.44 ± 0.04	18
d4T	47 ± 10	166 ± 13	3.5
ddI	44 ± 8	42 ± 7	1
3TC	11 ± 2	11 ± 4	1
EFdA	0.04 ± 0.02	0.13 ± 0.02	3.3

[a] Values are the mean ± standard deviation (SD) from four independent experiments and were determined using the one-site competition equation in GraphPad Prism 5.

To study the activation of AZT and d4T, we treated phorbol myristate acetate (PMA)-differentiated THP-1 and THP-$1_{KD\text{-}SAMHD1}$ cells with radiolabeled [^{14}C]AZT or [^{14}C]d4T, incubated the cells for 24 h at 37°C, lysed the cells, separated the NRTI metabolites by HPLC, and analyzed the metabolites with a liquid scintillation counter. We found an ~10-fold decrease in the amount of AZT-TP recovered from THP-$1_{KD\text{-}SAMHD1}$ cells, in comparison with THP-1 cells, but no significant difference in the amounts of d4T-TP recovered from the two cell lines (Fig. 2). These results are consistent with the observation that AZT experiences a larger EC_{50} increase than does d4T upon SAMHD1 knockdown, suggesting that AZT does not compete as well as d4T for phosphorylation by cellular kinases when there are increased levels of dNTPs and therefore it experiences a larger change in EC_{50}.

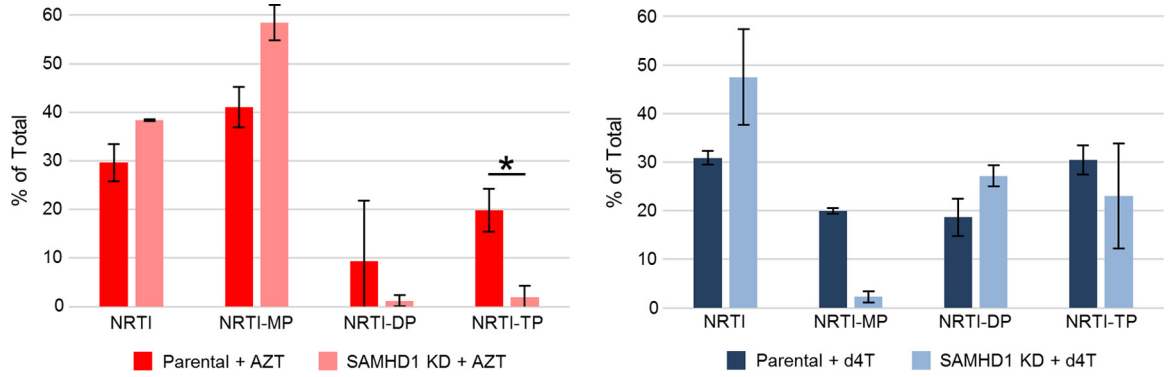
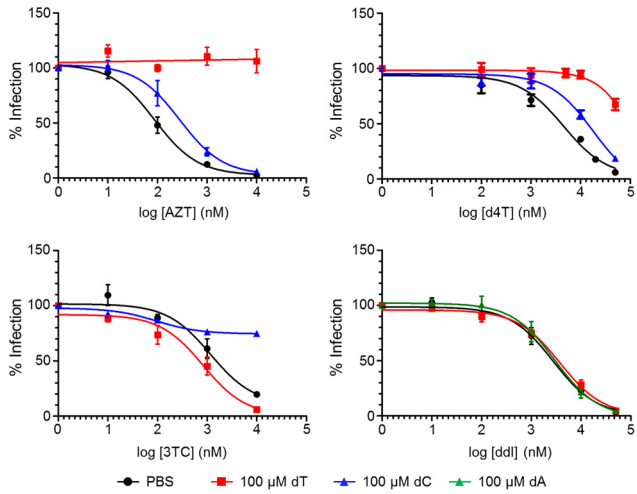


FIG 2 Knockdown of SAMHD1 has differential effects on NRTI activation. PMA-differentiated parental and THP-$1_{KD\text{-}SAMHD1}$ cells were treated with 1.5 μM (0.5 μCi) [2-^{14}C]AZT or [4-^{14}C]d4T. After 24 h at 37°C, the cells were lysed, the dNTP and NRTI metabolites were separated by anion-exchange HPLC (DNAPac PA100 column), and the NRTI metabolites in the collected fractions were quantified with a liquid scintillation counter. Data represent the mean ± standard deviation (SD) from 2 independent experiments. *, $P < 0.05$.

We directly explored the impact of increased cellular dTTP levels on the inhibitory potential of AZT and d4T by exogenously adding thymidine. We treated TZM-bl cells with phosphate-buffered saline (PBS) or 100 μM dT or dC (as a control as a noncompeting nucleoside) and infected the cells with HIV-$1_{NL4\text{-}3}$ (multiplicity of infection [MOI], 0.02) in the presence of increasing inhibitor concentrations. At 48 h postinfection, cells were lysed and luciferase activity was measured. As expected, exogenous dT increased the EC_{50}s for HIV-$1_{NL4\text{-}3}$ inhibition by AZT and d4T. Whereas the EC_{50} for AZT increased >100-fold upon addition of exogenous dT, the EC_{50} for d4T appeared to increase significantly less, although the exact EC_{50}s could not be estimated because we could not reach extremely high NRTI concentrations (Fig. 3). These data agree with our observation that SAMHD1 knockdown has a greater effect on AZT than on d4T. Notably, addition of 100 μM dA did not affect the ddI EC_{50} (Fig. 3), consistent with reported differences in the ddI and dA activation mechanisms (39, 44–48) and also with the lack of differences in EC_{50} values for ddI in THP-1 versus THP-$1_{KD\text{-}SAMHD1}$ cells (Table 1). While addition of 100 μM dC blocked HIV inhibition by 3TC (Fig. 3), the unchanged 3TC EC_{50} values in THP-1 and THP-$1_{KD\text{-}SAMHD1}$ cells may be partly attributed to the findings that SAMHD1 depletion had the smallest effect on the concentration of dCTP, compared to other dNTPs (19), and that 3TC-TP was a poorer substrate for SAMHD1 (Fig. 1).

We have demonstrated that SAMHD1 downregulation affects not only dNTP concentrations (3, 4, 14–19) but also the concentrations of AZT and d4T metabolites (Fig. 2). Our data are consistent with previous reports noting that the rate-limiting step in activation is the second phosphorylation step, catalyzed by thymidylate kinase, for AZT but the first phosphorylation step, catalyzed by thymidine kinase, for d4T (39, 49–54), as shown by the accumulation of AZT-MP and d4T in treated THP-1 and THP-$1_{KD\text{-}SAMHD1}$ cells (Fig. 3). d4T diphosphate (DP) is more readily phosphorylated to the triphosphate form by nucleoside diphosphate kinase, the final kinase in the activation pathway for d4T and AZT, as well as other analogs and deoxynucleoside diphosphates (dNDPs) (39, 55–58), than is AZT-DP, addressing why the potency of AZT is affected more than that of d4T with SAMHD1 depletion and increased dNTP concentrations.

In conclusion, the presence of SAMHD1, or its depletion, as occurs for lentiviruses that encode the Vpx accessory protein (12, 59–63), can affect NRTI susceptibility in multiple ways that depend not only on the relative changes in the concentrations of dNTPs and NRTI-TPs but also on the activation pathways of NRTIs. Our study highlights the importance of the metabolic



EC_{50} (μM)

Treatment	AZT	d4T	3TC	ddI
PBS	0.1 ± 0.05	5.4 ± 3.0	1.7 ± 0.9	2.2 ± 1.6
100 μM dT	> >10	> 50	0.9 ± 0.6	2.6 ± 1.2
100 μM dC	0.3 ± 0.18	13.2 ± 7.5	> 10	ND
100 μM dA	ND	ND	ND	2.2 ± 1.2
Fold Change	>>100	>10	> 6	1

FIG 3 Exogenously added dT, but not dC, affects AZT and d4T potencies. TZM-bl cells were treated with PBS, 100 μM dT, or 100 μM dC or 100 μM dA (as controls for noncompeting nucleosides) and infected with HIV-$1_{NL4\text{-}3}$ at an MOI of 0.02, in the presence of increasing concentrations of inhibitor (AZT or d4T). At 48 h postinfection, cells were lysed and luciferase activity was detected. Luciferase activity at various drug concentrations was plotted using the one-site competition equation in GraphPad Prism 5, and data were normalized to the no-nucleoside control results. Data represent the mean ± SD from at least three independent experiments. Data in the table represent the mean ± SD from at least three independent experiments. Shown also are the fold changes in the EC_{50} of NRTI in the presence or absence of cognate nucleoside, which indicate change in sensitivity to AZT/d4T, 3TC, or ddI, in the presence of dT, dC, or dA, respectively. ND, not determined.

pathways for activation of different NRTIs to NRTI-TPs, especially in cells in which dNTP concentrations are low and competition with NRTI-TPs does not mask the effects of differential NRTI activation.

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