Inhibitors of CDK2 and CDK6 Block HIV-1 RT through the Control of SAMHD1 Activity

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ABSTRACT

BACKGROUND: SAMHD1 inhibits HIV-1 reverse transcription by decreasing the pool of intracellular deoxynucleotides in myeloid and lymphoid cells. The activity of the HIV-1 restriction factor SAMHD1 is controlled by cyclin-dependent kinase (CDK)-mediated phosphorylation. However, the exact mechanism of SAMHD1 regulation in primary cells is unclear.

METHODOLOGY: Human primary monocytes obtained from PBMC were differentiated into macrophages with M-CSF. CD4+ T lymphocytes were obtained from PBMC and stimulated with PHA/IL-2. Cells were pre-treated with CDK inhibitors and then infected with a GFP-expressing HIV-1 or R5-tropic virus BaL. Proviral DNA formation was measured by quantitative PCR and infection assessed by flow cytometry. SAMHD1 was degraded treating cells with viral-like particles carrying Vpx (VLPVpx).

RESULTS: Pan CDK inhibitors roscovitine and purvalanol A show in vitro activity against several CDK, including CDK2 and AT7519 is a preferential inhibitor of CDK2. These CDK inhibitors reduced SAMHD1 phosphorylation in macrophages and CD4+ T lymphocytes as measured by Western blot analysis. HIV-1 replication was blocked in primary macrophages by roscovitine (47.3%±3.9; n=4), purvalanol A (55.7%±15.7; n=4) and AT7519 (66.4%±3.8; n=4) at subtoxic concentrations as measured by the colorimetric MTT assay. CDK inhibitors blocked the formation of proviral DNA formation, indicating an effect on the reverse transcription step. Similar results were obtained in primary CD4+ T lymphocytes. In addition to these broad-spectrum CDK inhibitors, we tested the antiviral activity of palbociclib, a potent and selective CDK6 inhibitor. Palbociclib potently inhibited SAMHD1 phosphorylation, HIV-1 reverse transcription and HIV-1 replication (99.5%±0.2; n=4). Notably, treatment of macrophages with palbociclib led to reduced CDK2 activation, measured as the phosphorylation of the T-loop at the Thr160. The antiviral effect of all CDK inhibitors was lost when SAMHD1 was degraded using VLPVpx, providing further evidence for a role of SAMHD1 in mediating the antiretroviral effect.

CONCLUSIONS: Our results indicate that SAMHD1-mediated restriction of HIV-1 infection is controlled by CDK as previously suggested, but point to a preferential role for CDK2 and CDK6 as mediators of SAMHD1 activation. Our study provides a new signalling pathway susceptible for the development of new therapeutic approaches against HIV-1 infection.

PAN CDK INHIBITORS (ROSCOVITINE, PURVALANOL A, AT7519) AND CDK6 INHIBITOR PALBOCICLIB REDUCE SAMHD1 PHOSPHORYLATION IN MDM AND LYMPHOCTES

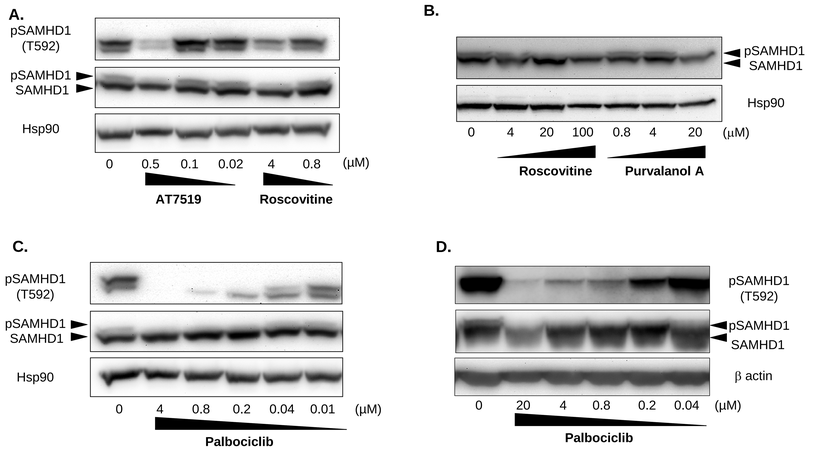


Figure 1. (A) and (B) Pan CDK inhibitors, Roscovitine, Purvalanol A and AT7519 reduce SAMHD1 phosphorylation in primary cells. Western blots of macrophages (A) and CD4+ T lymphocytes (B) untreated or treated with serial dilutions of AT7519 and Roscovitine for macrophages (A) and Rocovitine and Purvalanol A (B) for lymphocytes at indicated concentrations for 48 hr. Hsp90 antibody blot was used as loading control. One blot of three independent donors is shown. **(C) and (D) CDK6 inhibitor Palbociclib inhibits SAMHD1 phosphorylation in primary cells.** Western blots of macrophages (C) and CD4+ T lymphocytes (D) untreated or treated with serial dilutions of palbociclib at indicated concentrations for 48 hr. Hsp90 or β-actin antibodies blot were used as loading controls. One blot of three independent donors is shown.

BLOCKADE OF HIV-1 REPLICATION BY CDK INHIBITORS IS DEPENDENT ON SAMHD1 FUNCTION IN MDM

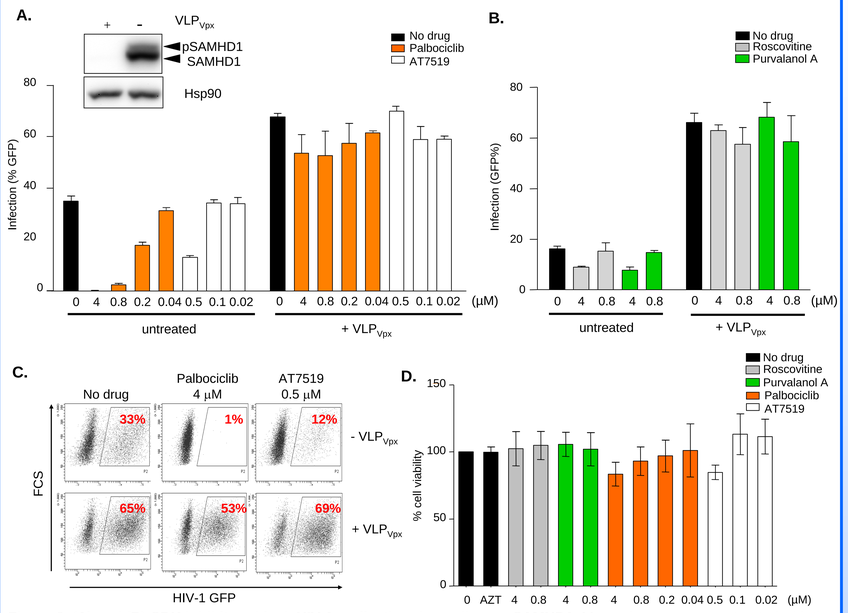


Figure 2. (A) and (B) CDK inhibitors block HIV-1 replication depending on SAMHD1. Macrophages were pre-treated with the indicated concentration of drugs in the absence (untreated) or presence of viral-like particles carrying Vpx (VLP_{Vpx}) and then infected with VSV-pseudotyped, GFP-NL4-3. Degradation of SAMHD1 by VLP_{Vpx} was checked by western blot (top left panel). Mean ± SD of infection triplicates of one donor out of five with similar results is shown. **(C)** Representative dot plot showing percentage of infected GFP positive cells measured by flow cytometry. **(D) Absence of toxicity of CDK inhibitors in macrophages.** MTT assay was performed in macrophages treated for two days with the indicated concentrations of drugs and 3 μM of AZT as a control. Values are normalized to the no drug sample. Mean ± SD of three independent donors are shown.

CDK INHIBITORS BLOCK HIV-1 REVERSE TRANSCRIPTION STEP

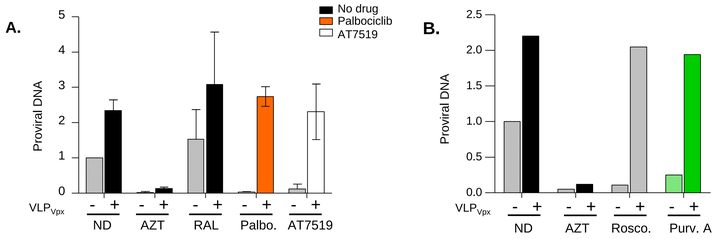


Figure 3. (A) and (B) Proviral DNA formation after 16hr infection with HIV-1 BaL of macrophages treated or not with VLP_{Vpx} in the presence of the indicated drugs, AZT (3 μM) or Raltegravir (RAL; 2 μM), Palbociclib (4 μM) and AT7519 (0.5 μM) in (A) and Roscovitine (4 μM) and Purvalanol A (4 μM) in (B). Data were normalized to proviral DNA detected in untreated macrophages (ND) without VLP_{Vpx}. Data represent mean±SD of three independent experiments (A) or a representative donor in (B).

CDK INHIBITORS BLOCKADE OF HIV-1 RT IS MEDIATED BY SAMHD1 IN CD4+ T LYMPHOCYTES

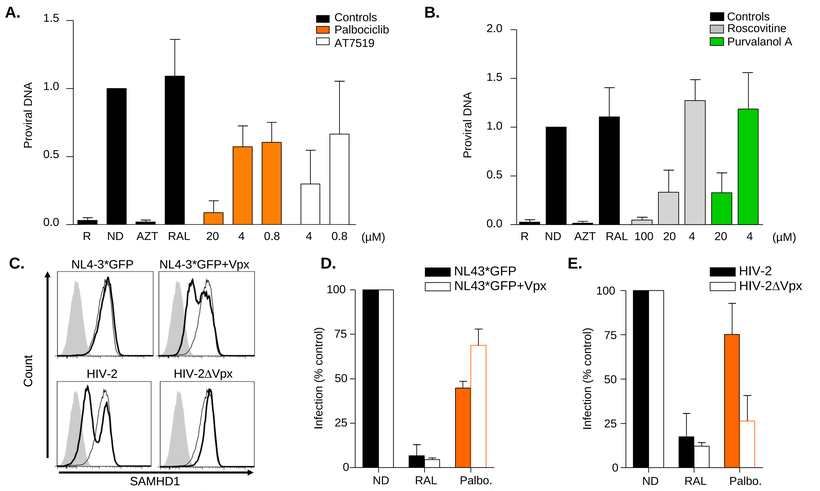


Figure 4. CDK inhibitors block HIV-1 reverse transcription in CD4+ T lymphocytes. (A) and (B) Proviral DNA 5h post-infection with HIV-1 NL4-3 of resting (R) or PHA/IL-2 stimulated CD4+ T cells pre-treated for 6 hr with AZT (3 μM), raltegravir (RAL; 2 μM), palbociclib or AT7519 in (A) and Roscovitine and Purvalanol A in (B) at indicated concentrations. Results were normalized to proviral DNA detected in untreated activated lymphocytes (ND). Data represent mean±SD of at least three independent experiments. **(C) Degradation of SAMHD1 by Vpx in lymphocytes.** Flow cytometry histograms showing intracellular staining of SAMHD1 in lymphocytes infected with an NL4-3*GFP carrying or not Vpx protein or HIV-2. **(D) and (E)** Activated CD4+ T lymphocytes were pre-treated without drug (ND) or with raltegravir (RAL; 1 μg/ml) or palbociclib (Palbo., 4 μM) and then infected with NL4-3*GFP carrying or not Vpx protein (D) or HIV-2 and HIV-2ΔVpx (E). Infections were normalized to the corresponding control in the absence of drug. Mean±SD of four independent donors are shown.

PALBOCICLIB TREATMENT LEADS TO REDUCED CDK2 ACTIVATION AND dNTP INTRACELLULAR POOL

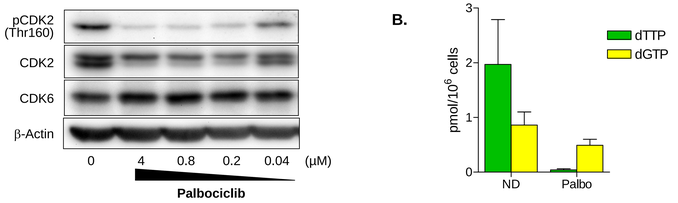


Figure 5. (A) Activation of CDK2 is inhibited in macrophages treated with Palbociclib. Western blot of untreated macrophages or treated with palbociclib at the indicated doses. Membranes were blotted with an anti phospho-CDK2 antibody raised against phospho-Thr160 (pCDK2(Thr160)). Anti-CDK2, anti-CDK6 and anti-β-actin antibodies were also used as a control. Representative blots of three independent experiments performed are shown. **(B) Intracellular dNTP levels are reduced after Palbociclib tratment in macrophages.** MDM were treated or not with Palbociclib (Palbo 4 μM) for 24 hours and intracellular dNTPs were extracted and dNTP content was determined using a polymerase-based method.

CONCLUSIONS

- SAMHD1-mediated restriction of HIV-1 infection is controlled by CDK as previously suggested.
- Our results point to a preferential role for CDK2 and CDK6 as mediators of SAMHD1 activation.
- Inhibition of CDK6 by palbociclib decreases intracellular dNTP pool, providing a link between SAMHD1 deactivation and HIV restriction.