Bystander Effect of Histone Deacetylase Inhibitors on HIV-1 Infection

Grant R. Campbell[1], Rachel S. Bruckman[1], Yen-Lin Chu[1], and Stephen A. Spector[1,2]

[1]Department of Pediatrics, Division of Infectious Diseases, University of California San Diego, La Jolla, California, USA, and [2]Rady Children's Hospital, San Diego, California, USA

Abstract

Background: Histone deacetylase inhibitors (HDACi) are being evaluated in a "shock-and-kill" therapeutic approach to reverse HIV latency from $CD4^+$ T cells. Although administered within the context of combination antiretroviral therapy (cART), infection of bystander cells remains a concern. In this study, we investigated the effect of HDACi on the replication kinetics of HIV within human primary macrophages.

Methods: Monocyte-derived macrophages (MDM) were treated with HDACi (belinostat, givinostat, panobinostat, romidepsin, or vorinostat) at therapeutic doses. The sequential steps of HIV infection and replication were assessed using flow cytometry for receptor expression, HIV p24 ELISA for binding, entry and release dynamics, qPCR for reverse transcription, and Alu-LTR qPCR for integration. The role of autophagy was investigated using small molecule inhibitors including bafilomycin A_1 or by RNAi for *ATG7* or *ATG5*. Cell toxicity was assessed by lactate dehydrogenase release and ssDNA accumulation. Data were analyzed using the Student's *t* test.

Results: None of the HDACi tested had an effect on viral binding, entry, reverse transcription, or integration. Moreover, HIV-replicative fitness post-HDACi treatment was unchanged. However, pre-treatment with HDACi induced a dose-dependent significant decrease in HIV p24 antigen release into the culture supernatants (romidepsin > panobinostat > vorinostat > givinostat > belinostat) ($P < 0.01$). Furthermore, HDACi exposure at the time of infection and at 3, 5, and 7 days post-infection resulted in a significant decrease in HIV p24 antigen ($P < 0.001$) in the absence of cytotoxicity. The inhibition of HIV by HDACi was significantly reduced using RNAi for *ATG5* or *ATG7* and small molecule inhibitors of autophagic flux ($P < 0.05$). Finally, all HDACi induced a significant decrease in intracellular HIV p24 antigen that was dependent upon autophago-lysosome fusion events indicating the degradation of HIV through autophagy.

Conclusions: All HDACi tested induced a dose-dependent degradation of intracellular HIV through the canonical autophagy pathway that requires the formation of autophagosomes and their subsequent maturation into autolysosomes. In contrast, HDACi were found to have no effect on initial infection events including viral entry, binding, and integration. These findings demonstrate that HDACi have important off-target effects that should be considered when being used as part of a cure strategy.

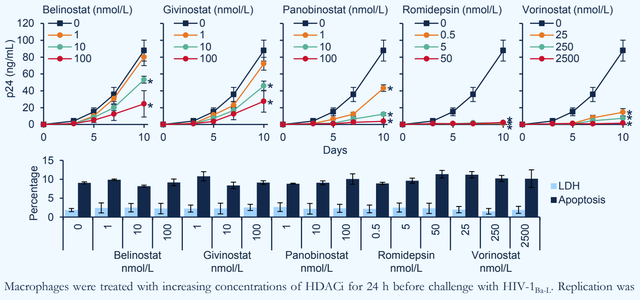
Background

- HDACi are being evaluated in a "shock-and-kill" therapeutic approach to reverse HIV latency.
- Although administered within the context of cART, the infection of bystander cells remains a concern.
- Vorinostat has been shown to increase the kinetics of post-entry events such as reverse transcription and integration thereby promoting productive infection of $CD4^+$ T cells and potentially reseeding the viral reservoirs being purged.
- No study has examined the effect of HDACi on viral infectivity of macrophages.
- Autophagy is a highly conserved degradation pathway whereby autophagosomes engulf cytoplasmic constituents, such as microbial pathogens, fuse with lysosomes, and degrade the engulfed components.

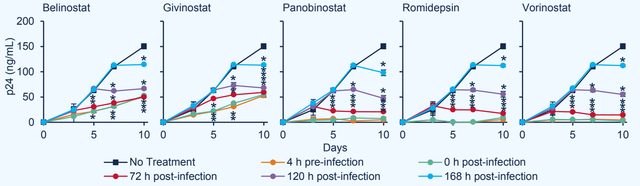
Results

Figure 1

HDACi inhibit HIV-1 infection in macrophages



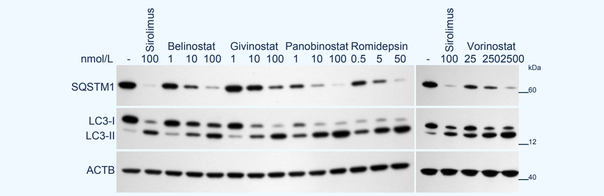
Macrophages were treated with increasing concentrations of HDACi for 24 h before challenge with HIV-$1_{Ba\text{-}L}$. Replication was followed using HIV p24 expression. All HDACi inhibited HIV-1 in a dose-dependent manner in the absence of cytotoxicity. Only the lowest doses of belinostat and givinostat failed to significantly inhibit HIV-1 replication. * $P < 0.05$, $n = 5$.



HDACi decrease HIV p24 release post-infection. Macrophages were incubated with HDACi at different time points with respect to infection with HIV. ELISAs were performed for HIV p24 antigen over 10 d. All HDACi were used at 100 nmol/L except romidepsin which was used at 50 nmol/L. Results are reported as mean ± s.e.m., $n = 6$; * $P < 0.05$.

Figure 2

HDACi induce autophagy in macrophages



Macrophages were treated with HDACi or the MTOR inhibitor sirolimus (a positive control) for 24 h. The degradation of sequestosome 1 (SQSTM1), and microtubule-associated protein 1 light chain 3B (LC3B) lipidation were analyzed. SQSTM1 binds LC3B-II and is degraded when autophagosomes fuse with lysosomes. All HDACi induced the dose-dependent induction of autophagy. *Top*, a representative blot. *Bottom*, densitometric analysis from 4 independent donors. * $P < 0.05$; n = 4.

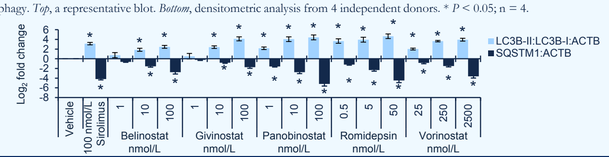
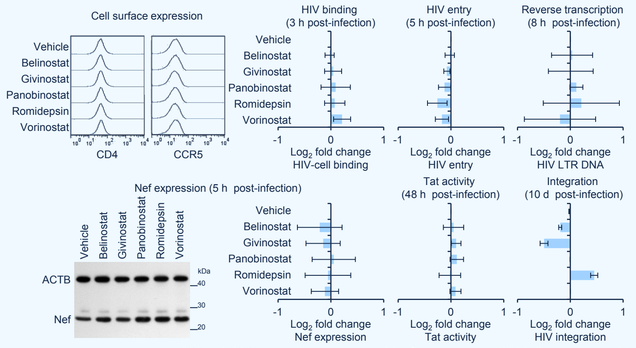
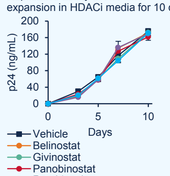


Figure 3

4 h pre-treatment with HDACi does not affect HIV replication



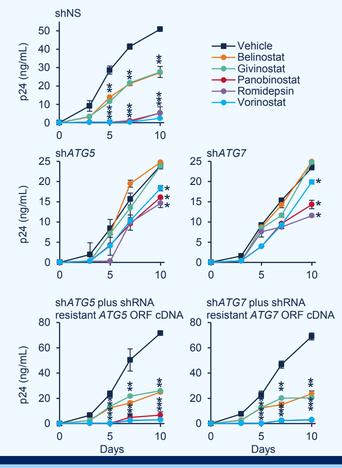
Replicative ability of HIV after expansion in HDACi media for 10 d

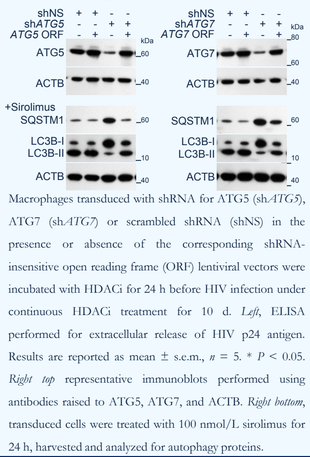


CD4 and CCR5 expression was measured by flow cytometry. HIV binding was assessed by washing cells, lysing, and analyzing p24 by ELISA. Entry was measured by washing cells, trypsinization, lysing, and analyzing intracellular p24 by ELISA. Reverse transcription was analyzed by qPCR of pre-integration strong-stop HIV LTR DNA. Nef expression was analyzed by Western blotting. Tat activity was measured using TZM-bl cells. Integration was assessed using an Alu-LTR-based nested qPCR. Replicative ability of HIV post-HDACi treatment was assessed by exposing uninfected macrophages to 1 ng HIV p24 antigen from the 10 d aliquots of cell-free supernatants post-HDACi treatment for 10 d. ELISA performed for p24. All HDACi were used at 100 nmol/L except romidepsin which was used at 50 nmol/L. Results are reported as mean ± s.e.m., $n = 5$. * $P < 0.05$.

Figure 4

HDACi inhibition of HIV-1 requires autophagosome formation

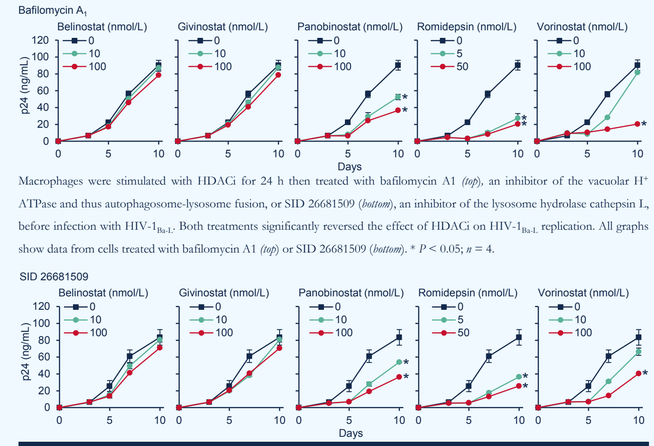




Macrophages transduced with shRNA for ATG5 (sh*ATG5*), ATG7 (sh*ATG7*) or scrambled shRNA (shNS) in the presence or absence of the corresponding shRNA-insensitive open reading frame (ORF) lentiviral vectors were incubated with HDACi for 24 h before HIV infection under continuous HDACi treatment for 10 d. *Left*, ELISA performed for extracellular release of HIV p24 antigen. Results are reported as mean ± s.e.m., $n = 5$. * $P < 0.05$. *Right top* representative immunoblots performed using antibodies raised to ATG5, ATG7, and ACTB. *Right bottom*, transduced cells were treated with 100 nmol/L sirolimus for 24 h, harvested and analyzed for autophagy proteins.

Figure 5

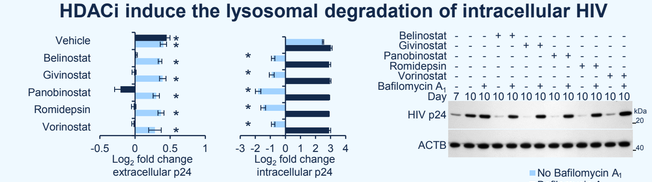
HDACi inhibit HIV-1 through the induction of autophagic flux



Macrophages were stimulated with HDACi for 24 h then treated with bafilomycin A1 *(top)*, an inhibitor of the vacuolar H^+ ATPase and thus autophagosome-lysosome fusion, or SID 26681509 *(bottom)*, an inhibitor of the lysosome hydrolase cathepsin L, before infection with HIV-$1_{Ba\text{-}L}$. Both treatments significantly reversed the effect of HDACi on HIV-$1_{Ba\text{-}L}$ replication. All graphs show data from cells treated with bafilomycin A1 *(top)* or SID 26681509 *(bottom)*. * $P < 0.05$; $n = 4$.

Figure 6

HDACi induce the lysosomal degradation of intracellular HIV



Macrophages were infected for 7 d with HIV then treated with HDACi in the presence or absence of bafilomycin A_1 for 3 d. All HDACi were used at 100 nmol/L except romidepsin which was used at 50 nmol/L. *Top*, ELISA was performed for both extracellular and intracellular HIV p24 antigen at 7 and 10 d post-infection. $n = 3$. *Right top*, representative immunoblots of HIV p24 antigen using antibody to HIV p24 antigen and ACTB. *Right bottom*, densitometric analysis of immunoblots presented as means ± s.e.m., $n = 4$. * $P < 0.05$.

Conclusions

- HDACi do not promote the productive infection of macrophages
- HDACi trigger autophagy
- Belinostat, givinostat and vorinostat, and to a lesser extent, panobinostat and romidepsin inhibition of HIV-1 is dependent upon autophagic degradation of HIV

Acknowledgements

This work was supported by the NINDS (R01 NS084912), the NIAID, NIH (AI084573), the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network and the California HIV/AIDS Research Program (ID12-SD-255).

University of California San Diego, Stein Clinical Research Building Room 432, 9500 Gilman Drive, La Jolla CA 92093-0672, USA • gcampbell@ucsd.edu saspector@ucsd.edu • Phone: +1 (858) 534 7477 • Fax: +1 (858) 534 7411