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Latent HIV-1 Reactivation and Lysosomal Destabilization Synergize to Host Cell Death

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ABSTRACT

Background: Modern strategies for purging the latent HIV-1 reservoir use pharmacological reactivation followed by elimination of cells that reactivated viral latency either by the virus or/and patient's immune system 'shock and kill'. Unfortunately, most of the current approaches demonstrated only partial reactivation and limited reduction of the reservoir. We aimed to develop a pharmacological strategy targeting cellular pro-survival mechanisms in order to sensitize and eliminate host cells with even low or incomplete levels of HIV-1 reactivation.

Methods: We used *in vitro* models of HIV latency (J-lat full length clones, ACH2 and U1) and the latency-reversing agent panobinostat (Pan). We targeted the pro-survival pathway of autophagy and lysosomal integrity by the lysosome-destabilizing agents (LDA) chloroquine (CQ) or mefloquine (Mef). Autophagy and lysosomal pathways were monitored using flow cytometry, fluorescent microscopy and western blotting. Latent HIV-1 reactivation was confirmed through HIV-1-GFP expression, intracellular p24 abundance, and secretion.

Results: Our results confirmed that CQ and Mef suppress autophagic flux and destabilize lysosomal membrane. Pan mediated reactivation led to specific cell death, which closely correlated to the levels of reactivation (R^2=0.8821; p=0.0178). At Pan concentrations associated with HIV-1 reactivation, but only little host cell death, co-treatment with CQ increased the death rate about four fold (Control 1.71%; Pano 17.4%; CQ 10% and Pano+CQ 63.2% *p<0.0001*). These synergistic and selective effects were further enhanced and were more rapid when we combined lysosomal disruption by Mef with boosted lysosomal activity by partial nutrient reduction (Control 9.2%; Pano 23.6%; Mef 8.8%, RPMI 13.3 and Pano+Mef+RPMI 74.7% *p<0.0001*).

Conclusions: We conclude that interference with the pro-survival pathway of autophagy at the level of lysosomal stability efficiently supports host cell death upon incomplete HIV-1 reactivation. These *in vitro* results suggest that combined pharmacological intervention may assist to eradicate latently HIV-1 infected cells.

INTRODUCTION

Modern strategies for purging the latent HIV-1 reservoir use pharmacological reactivation followed by elimination of cells that reactivated viral latency either by the virus or/and patient's immune system 'shock and kill'. Unfortunately, most of the current approaches demonstrated only partial reactivation and limited reduction of the reservoir.

OBJECTIVE

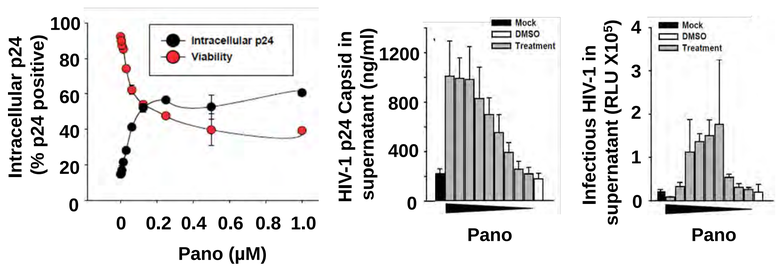
We aimed to develop a pharmacological strategy targeting cellular pro-survival mechanisms in order to sensitize and eliminate host cells with even low or incomplete levels of HIV-1 reactivation.

METHODS

We used *in vitro* models of HIV latency (J-lat full length clones, ACH2 and U1) and the latency-reversing agent panobinostat (Pan). We targeted the pro-survival pathway of autophagy and lysosomal integrity by the lysosome-destabilizing agents (LDA) chloroquine (CQ) or mefloquine (Mef). Autophagy and lysosomal pathways were monitored using flow cytometry, fluorescent microscopy and western blotting. Latent HIV-1 reactivation was confirmed through HIV-1-GFP expression, intracellular p24 abundance, and secretion.

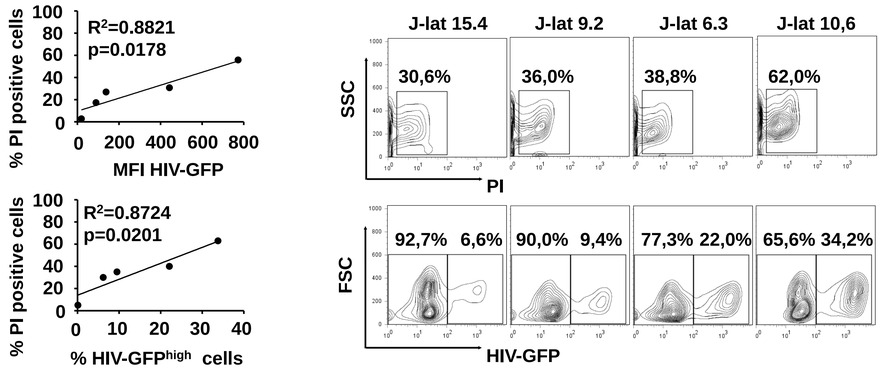
RESULTS

HDACi induced HIV-1 reactivation is associated with increased host cell death

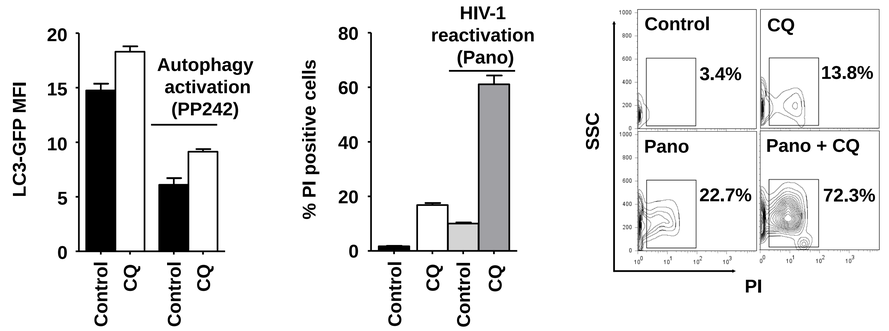


RESULTS

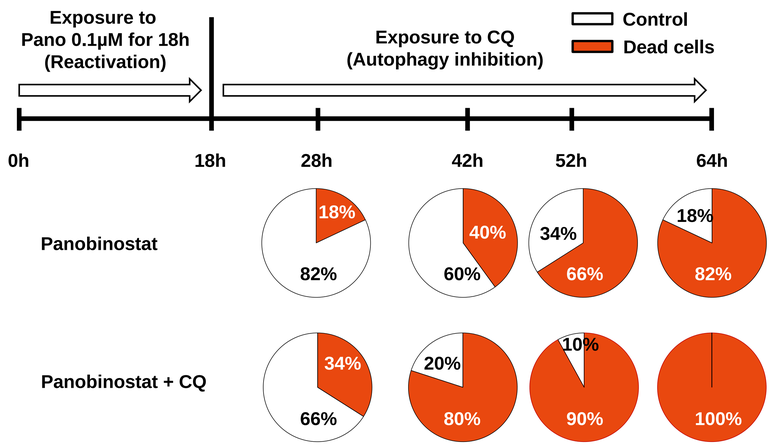
Level of cell death correlates with level of reactivation



CQ suppresses autophagy and supports cell death in reactivated cells

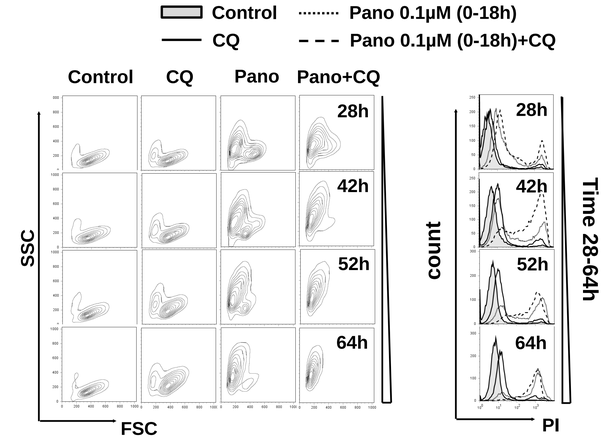


In vitro modeling of latent HIV-1 eradication

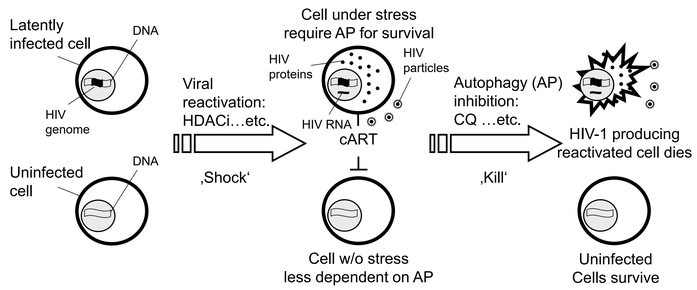


RESULTS

In vitro model of latent HIV-1 eradication



Model of selective eradication



CONCLUSION

Interference with the pro-survival pathway of autophagy at the level of lysosomal stability efficiently supports host cell death upon incomplete HIV-1 reactivation. These *in vitro* results suggest that combined pharmacological intervention may assist to eradicate latently HIV-1 infected cells

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