Reactivation of latent HIV-1 by new semi-synthetic ingenol esters


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ABSTRACT
The ability of HIV to establish long-lived latent infection is mainly due to transcriptional silencing of viral genome in resting memory T lymphocytes. Here, we show that new semi-synthetic ingenol esters reactivate latent HIV reservoirs. Amongst the tested compounds, 3-caproyl-inganol (ING B) was more potent in reactivating latent HIV than known activators such as SAHA, ingenol 3,20-dibenzotate, TNF-α, PMA and FK506. ING B activated PKC isozymes followed by NF-kB nuclear translocation. As virus reactivation is dependent on intact NF-kB binding sites in the LTR promoter region, we have shown that ING B was able to reactivate virus transcription in primary HIV-infected resting cells up to the 12-fold and up to 25-fold in combination with SAHA. Additionally, ING B promoted up-regulation of P-TF Eb subunits CDK9/Cyclin T1. The role of ING B on promoting both transcription initiation and elongation makes this compound a strong candidate for an anti-HIV latency drug combined with suppressive HAART.

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Introduction
The eradication of HIV by current anti-retroviral therapies in infected individuals is not accomplished due to the establishment of viral latency during early stages of infection. HIV establishes long-lived latent infection in resting memory T lymphocytes and other non-dividing cells mainly due to transcriptional silencing (Finzi et al., 1997; Geeraert et al., 2008). Despite undetectable viral load in patients treated with potent antiretroviral therapy, proviral genomes remain in the latent reservoirs rendering the total clearance of HIV an unattainable goal at present (Finzi et al., 1997; Pierson et al., 2000; Yang et al., 2009). Hence, the search for a cure is one of the most challenging and rewarding areas of HIV/AIDS research (Geeraert et al., 2008; Johnston, 2010).

Multiple mechanisms contribute to the maintenance of HIV latency, including integration of the proviral DNA in transcriptionally inactive sites, Tat trans-activation, histone modifications or unavailability of cellular transcription factors (Ganesh et al., 2003; Ishida et al., 2006; Jones and Peterlin, 1994; Mbonye and Karn, 2014; Williams et al., 2006). Additionally, post-transcriptional mechanisms affecting the export or translation of HIV mRNAs can also block HIV expression during latency (Huang et al., 2007; Karn and Stoltz, 2012; Lassen et al., 2006). HIV activation and replication is highly dependent on T lymphocyte activation status, relying on the availability of host transcription factors, such as NF-kB, NFAT and AP1, which bind to HIV-1 LTR triggering virus RNA transcription, and others such as P-TF Eb that act in transcriptional elongation (Bartholomeeusen et al., 2013; Contreras et al., 2012, 2007; Gonzalez et al., 2003; Khalaf et al., 2010; Kinoshiba et al., 1997; Mbonye and Karn, 2014; Sgarbanti et al., 2008; Torgerson et al., 1998; Yang et al., 1999). Protein kinase C (PKC) activation followed by NF-kB nuclear translocation is an important step required for latent HIV reactivation.

The PKC family comprises a large number of serine-threonine kinases dependent on the hydrolysis of phosphatidyl-inositol-4, 5-bisphosphate (PIP2) for activation. PIP2 is hydrolyzed in diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3), promoting...