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Treatment of chronically FIV-infected cats with suberoylanilide hydroxamic acid

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ABSTRACT

Feline immunodeficiency virus (FIV) is a naturally-occurring, large animal model of lentiviral-induced immunodeficiency syndrome, and has been used as a model of HIV pathogenesis and therapeutic interventions. HIV reservoirs in the form of latent virus remain the primary roadblock to viral eradication and cure, and FIV has been previously established an animal model of lentiviral latency. The goal of this study was to determine whether administration of the histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA) to aviremic, chronically FIV-infected cats would induce latent viral reactivation *in vivo*. A proof-of-concept experiment in a Transwell co-culture system demonstrated the ability of SAHA to reactivate latent virus which was replication competent and able to infect naïve cells. Oral SAHA (250 mg/m^2) was administered with food to four asymptomatic, experimentally FIV-infected cats and one uninfected control cat, and a limited pharmacokinetic and pharmacodynamic analysis was performed. A statistically significant increase in cell-associated FIV RNA was detected in the cat with the greatest serum SAHA exposure, and cell-free viral RNA was detected at one time point in the three cats that achieved the highest levels of SAHA in serum. Interestingly, there was a significant decrease in viral DNA burden at 2 h post drug administration in the same three cats. Though the sample size is small and the drug response was modest, this study provides evidence that *in vivo* treatment of FIV-infected cats with the HDACi SAHA can induce viral transcriptional reactivation, which may be dependent upon the concentration of SAHA achieved in blood. Importantly, alternative putative antilatency therapy drugs, and multimodal drug combinations, could be studied in this *in vivo* system. The FIV/cat model provides a unique opportunity to test novel therapeutic interventions aimed at eradicating latent virus *in vivo*.

1. Introduction

Feline immunodeficiency virus (FIV) is a lentivirus of domestic cats with a seroprevalence of approximately 1% among asymptomatic cats in the United States. Seroprevalence is much higher in symptomatic animals, and in countries such as Japan and Australia, has been as high as 30% of the total population (Courchamp and Pontier, 1994). Terminally, infected animals exhibit feline acquired immunodeficiency syndrome (FAIDS), which includes opportunistic infections, tumor formation, wasting, and death. Though some antiretroviral therapies have been shown to be effective against FIV *in vitro*, FIV-infected cats are typically managed clinically by isolation, symptomatic treatment, and/or euthanasia. Although multiple antiretroviral agents are known to be effective against FIV (Bisset et al., 2002; Fogle et al., 2011), lifelong antiretroviral therapy is not financially or logistically feasible for the owners of most cats.

FIV is similar to the human immunodeficiency virus (HIV) in genome structure and immunopathogenesis (Burkhard and Dean, 2003; Kanzaki and Looney, 2004), and has been utilized as the only naturally occurring animal model of immunodeficiency for HIV-infection in people (Elder et al., 2010). During highly active antiretroviral therapy (HAART), HIV persists in infected individuals as a transcriptionally inactive (latent) integrated provirus (Trono et al., 2010), which prevents viral eradication and necessitates lifelong adherence to therapy. Memory CD4+ T cells are the primary long-lived lentiviral reservoir (Blankson et al., 2002; Chomont et al., 2009). Multiple molecular mechanisms may underlie the establishment and maintenance of latently-infected cellular reservoirs (Margolis, 2010), including epigenetic modification of histone proteins in chromatin (Colin and Van Lint, 2009; Imai et al., 2010; Margolis, 2010, 2011). FIV has been established as an outbred, large animal model of lentiviral latency, reviewed in (McDonnel

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et al., 2013). Studies in our laboratory demonstrated that domestic cats experimentally infected with FIV exhibit a state of latency in peripheral blood CD4+ T-cells with concurrent undetectable plasma viremia approximately 8 months after inoculation (Murphy et al., 2012). We have also shown that the latent, transcriptionally inactive FIV promoter persists within *in vivo*-derived CD4+ T-cells in association with de-acetylated histones, consistent with a repressive chromatin structure (McDonnel et al., 2012a). FIV infection of its natural host can therefore provide critical insight into generalizable mechanisms of lentiviral latency and allow for the investigation of novel therapeutics targeting latent viral reservoirs.

Histone modifications, especially by acetylation, play an important role in the epigenetic regulation of gene expression. Augmented histone acetylation results in a "relaxed" chromatin state, allowing binding of transcription factors and polymerases, and ultimately promotes transcription of RNA. Conversion from the acetylated to the deacetylated state of chromatin is accomplished by a family of enzymes called histone deacetylases (HDAC). Suberolyanilide hydroxamic acid (SAHA) is a potent small-molecule inhibitor of HDAC classes I, II and IV (Lane and Chabner, 2009), which include all prominent active nuclear HDACs in mammalian cells. Thus, SAHA is considered a pan-HDAC inhibitor, and has been shown to cause an overall increase in global histone acetylation.

In 2006, SAHA (vorinostat, Zolinza™) became the first HDAC inhibitor (HDACi) approved by the Food and Drug Administration as a treatment for cutaneous T-cell lymphoma (CTCL) in humans. In addition to use for treatment of CTCL and other cancers, there is much interest in using HDACi such as SAHA to eradicate HIV from infected individuals. SAHA would be used as an inducer of viral promoter-associated histone acetylation, thereby reactivating viral transcription. The proposed concept is to activate transcription of latently integrated provirus in the presence of antiretroviral drugs, so as to induce death of infected cells by either direct cytopathic effect of the virus or immune surveillance, but protect uninfected cells from *de novo* infection (Richman et al., 2009). In theory, this strategy would purge the latent reservoir and eliminate the need for life-long HAART (Geeraert et al., 2008). We have shown in our lab that SAHA is effective in reactivating FIV transcription, protein expression, and infectious particle assembly *ex* vivo (McDonnel et al., 2012b). We have also examined the pharmacokinetics and pharmacodynamics of oral and intravenous-administered SAHA in cats (McDonnel et al., 2014).

Use of the FIV-infected cat model of lentiviral latency may help address questions that are either logistically or ethically not feasible in HIV-infected humans, including the investigation of histone-modifying agents and other novel therapeutic antilatency approaches (McDonnel et al., 2013). Importantly, these findings have the potential to benefit FIV-infected cats as well as HIV-infected humans. In this study, we sought to determine whether a candidate HDACi (SAHA) would be able to induce activation of viral transcription and replication *in vivo*. We hypothesized that treatment of aviremic, chronically FIV-infected cats with oral suberoylanilide hydroxamic acid (SAHA) would result in viral reactivation and subsequent viremia.

2. Materials and methods

As a proof of concept experiment, freshly isolated peripheral blood mononuclear cells (PBMCs) from two chronically FIV-infected cats (#165 and 187) were co-cultured *ex vivo* in a Transwell system (Fig. 1c) with specific pathogen free (SPF) PBMC in the upper culture well separated by a 400 nM permeable polycarbonate membrane (Corning). Cells were cultured in peripheral blood leukocyte (PBL) media (Murphy et al., 2012) alone, or media containing 1 μM SAHA, SAHA and allogeneic feline SPF PBMC (*i.e.*, the same PBMC that were used in the upper well), or SAHA, SPF PBMC, and 5 μg/mL concanavalin A. One million cells from the upper well were sampled at 7 and 14 days post *ex vivo* culture, and assessed for the presence of FIV *gag* DNA using real-time PCR as previously described (Murphy et al., 2012), indicating a productive viral infection. Cellular GAPDH DNA was used to normalize the amount of viral DNA in each sample.

Four experimentally FIV-C infected cats (#165, 184, 186, and 187) between 4 and 4.5 years post viral inoculation and one sham-inoculated cat (#185) were administered 250 mg/m^2 SAHA (>98% pure, Cayman Chemical) orally in gelatin capsules. All cats were fasted overnight and offered food (1/4 can feline Hills A/D® in addition to their normal meal) approximately 10 min before oral drug administration. Peripheral blood was collected by venipuncture the day before (0, pre-dose), and 1, 2, 4, 8, and 24 h, as well as 5 days post administration into two tubes: one tube containing EDTA (for plasma collection and leukocyte isolation) and one lacking anticoagulant (for serum collection).

Serum [SAHA] was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS), and histone acetylation status was quantified by Western blotting of PBMC lysates, as described previously (McDonnel et al., 2012c, 2014; Patel et al., 2008). PBMC were isolated from EDTA-anti-coagulated blood by ficoll-hypaque, and cellular DNA and RNA were extracted using a commercial kit (Qiagen AllPrep Mini kit). Viral RNA (vRNA) was isolated from double-spun (Murphy et al., 2012) plasma using another commercial kit (Qiagen viral RNA kit), and both plasma and cell-associated RNA were DNAse treated (Ambion) and reverse transcribed (Origene). Copies of FIV *gag* and cellular GAPDH were quantified by real-time PCR using SYBR Mastermix (5 Prime) as previously described (Murphy et al., 2012). For plasma co-culture assays, 100 μL of double-spun plasma from 0, 1, and 2 h post SAHA administration was incubated for 7 days with 200,000 specific pathogen free feline PBMC in media containing mitogens as previously described (c). DNA was then isolated as above, and viral infection was detected by the presence of FIV *gag* DNA using real-time PCR.

3. Results

In the *ex vivo* study, FIV-infected PBMC isolated from two cats were co-cultured in a Transwell system with SPF feline PBMC, using various added conditions to illicit viral reactivation. Infectious virus was released from the PBMC of both cats, and able to infect SPF PBMC through the permeable membrane (Fig. 1). The addition of SAHA, allogeneic PBMC (to induce a mixed-lymphocyte reaction), and mitogen (concanavalin A) increased the viral DNA load in the naïve PBMC, indicating that these conditions augmented viral reactivation *ex vivo*. Because the mitogen is freely diffusible across the Transwell membrane, the possibility exists that the increased infectivity when adding this agent was due in part to its proliferative effects on recipient cells in the upper well.

Following oral SAHA administration in the *in vivo* study, drug concentrations in serum were evaluated by LC-MS/MS. Serum SAHA kinetics were found to be variable, though the limited sample time points precluded a complete analysis of pharmacokinetics. One cat, #165 had an observed maximum concentration, C_{max} of less than 25% of the next lowest C_{max} and more than an order of magnitude less than the highest C_{max} (63 nM). Cat 165 was presumed to have spit out or regurgitated part or all of the capsule (though no vomitus or capsule material was found). The remaining cats' observed C_{max} ranged from 250 to 840 nM, with an average of 460 nM (Fig. 2). It should be noted that the observed C_{max} is not

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