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Reduced human herpesvirus-8 oropharyngeal shedding associated with protease inhibitor-based antiretroviral therapy

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ABSTRACT

Background: Human herpesvirus 8 (HHV-8) replication increases the risk of Kaposi sarcoma (KS). Highly-active antiretroviral therapy (HAART) reduces the incidence of KS, and regimens that contain protease inhibitors (PIs) may be particularly effective.
Objective: To determine whether PI-based HAART regimens may more effectively inhibit HHV-8 shedding compared to regimens without PIs.
Study design: Prospective, observational study of 142 HIV-1 and HHV-8 co-infected men conducted in Seattle, Washington. Quantitative HHV-8 PCR testing was performed on daily swabs of the oropharynx, the primary site of HHV-8 replication. Associations between antiretroviral regimen and detection of HHV-8 DNA in swabs were evaluated using generalized estimating equations.
Results: HHV-8 DNA was detected in 3016 (26%) of 11,608 specimens collected. PI-based HAART was associated with a statistically significantly lower frequency of detection (RR 0.2; 95% CI 0.1–0.5) compared to ART-naïve persons, whereas HAART without a PI was not (RR 0.7; 95% CI 0.4–1.3). Compared to ART-naïve persons, there was also a trend toward lower quantities of HHV-8 detected during treatment with HAART regimens that contained a PI. These associations between PIs and measures of HHV-8 shedding could not be attributed to use of nelfinavir, which inhibits HHV-8 replication *in vitro*, and were independent of CD4 count and HIV plasma viral load (VL).
Conclusions: HAART regimens that contain PIs appear to decrease HHV-8 shedding compared to NNRTIs. Further study of PI-based HAART is warranted to determine the optimal regimens for prevention and treatment of KS.

1. Background

Kaposi sarcoma (KS) is an AIDS-defining malignancy caused by infection with human herpesvirus 8 (HHV-8). The rising incidence of KS in the United States leveled off in 1987 shortly after approval of zidovudine for antiretroviral therapy (ART), and decreased further after the widespread use of combination "highly active" antiretroviral therapy (HAART; three or more drugs with at least one protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI)) in 1995 [1]. Nevertheless, KS remains the most common malignancy among HIV-infected people worldwide, and incident cases develop even among patients on HAART whose HIV infection is well controlled [2–4]. Furthermore, although HAART is beneficial for the treatment of AIDS-KS, nearly half of patients will have persistent disease despite receiving the

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current standard of care [5–9]. As such, better KS prevention and treatment strategies are needed.

The mechanisms by which ART affects KS have not been fully defined. HHV-8 replication is a strong risk factor for the development of KS [10–16], and the use of ART is associated with reductions of HHV-8 levels in blood [17–19]. The immune reconstitution that accompanies effective ART likely improves immune control of HHV-8 replication and tumor surveillance [20,21]. Additionally, ART may interfere with KS progression by reducing the levels of the HIV-1 Tat protein, which has angiogenic and tumorigenic functions [22] and promotes replication of HHV-8 *in vitro* [23].

Several observational studies have suggested that PI-based HAART may be superior to NNRTI-based regimens for the treatment of prevention of KS, independently of effects on HIV plasma viral load (VL) or CD4 count [15,24–26]. However, this has not been found in all cohorts [27–29] and data from controlled trials with adequate power to address the question are not currently available [30]. Among their many cellular effects, various PIs display anti-angiogenic and anti-tumor properties that may impair the growth and persistence of KS lesions [31–33]. Furthermore, some antiretroviral drugs may have direct effects on HHV-8 replication. Among PIs, nelfinavir appeared to preferentially inhibit production of infectious HHV-8 *in vitro* at concentrations achieved in plasma with routine oral dosing [34]. Though an effect on HHV-8 replication by nucleoside reverse transcriptase inhibitors (NRTIs) has not been demonstrated, the HHV-8 thymidine kinase is capable of phosphorylating both zidovudine and stavudine [35,36]. As such, specific antiretroviral regimens may have activity against HHV-8 that could confer clinically important effects.

Men co-infected with HIV and HHV-8 frequently shed HHV-8 DNA in saliva, and daily collection of oropharyngeal swabs offers a detailed portrait of HHV-8 oropharyngeal replication [37]. Additionally, ART use is associated with a significantly reduced risk of HHV-8 oropharyngeal shedding [38]. We therefore examined HHV-8 shedding among HIV/HHV-8 co-infected men to determine whether the type of ART regimen or use of PIs affects HHV-8 oropharyngeal replication.

2. Study design

Study participants. Men in Seattle, Washington were recruited from outpatient clinics and advertisements in the community for participation in studies of the epidemiology of human herpesviruses between 1993 and 2009. All participants in one or more of these studies were included in the analyses described here if they met the inclusion criteria of: (1) a positive HIV-1 serology test, and (2) a positive test for HHV-8 infection by either serology or PCR. Participants were not assigned ART by study investigators, but rather were asked to record ART regimens prescribed by their HIV care providers.

Specimen and data collection. Oropharyngeal sampling was performed by participants, by swabbing the buccal, lingual, palatine and tonsillar mucosa in a standardized fashion using a Dacron swab, as previously described [39,40]. Swabs were collected during "sessions"; each session consisted of a period of consecutive days on which oral swab collection was performed. Some men participated in more than one session. The shedding rate was computed as the number of swabs in which HHV-8 DNA was detected by PCR divided by the number of swabs collected for each session. Blood was collected at the beginning of each session for measurement of HIV-1 plasma RNA and CD4T cell counts.

Laboratory testing. Whole virus enzyme immunoassay (EIA) or immunofluorescence assay (IFA) was used to detect serum antibodies to HHV-8 as previously described [41]. DNA was extracted from oral swabs HHV-8 DNA was measured quantitatively with a real-time fluorescent polymerase chain reaction (PCR) with primers to the *orf73* gene, with positive and negative controls as previously described [42,43]. Oral swabs with ≥150 copies were considered positive for HHV-8 [40]. CD4T cell counts were measured with flow cytometry and HIV-1 plasma RNA was quantified using the AMPLICOR Monitor HIV-1 Test (Roche, Alameda, CA).

Table 1
Characteristics of study population.

Characteristic	Total (n = 142)
Men, n (%)	142 (100)
Age (years), median (range)	42 (22–68)
Race, n (%)	
Non-white	27 (19)
White	115 (81)
Years since HIV diagnosis, median (range)	10 (0–24)
CD4T cell count (cells/μL), median (range)	385 (1–1240)
HIV plasma RNA copies/mL, n (%)	
<500	61 (46)
500–10,000	24 (18)
>10,000	47 (36)
Missing	10
Kaposi sarcoma, n (%)	
Yes	12 (8)
No	130 (92)

Statistical analysis. Participant characteristics, HHV-8 oropharyngeal detection patterns, and ART use were reviewed using descriptive statistics. The distribution of copies of HHV-8 DNA was highly skewed and thus $\log_{10}$-transformed prior to analyses. Correlates of HHV-8 shedding frequency were examined using generalized estimating equation (GEE) models with a Poisson link and robust standard errors to account for overdispersion, and correlation among multiple sessions belonging to the same participant [44]. Analyses of the quantity of HHV-8 detected among sessions with at least one day with HHV-8 detected were performed using GEE models for normal outcomes [44]. HAART was defined as at least a three-drug regimen that included either a PI or a non-nucleoside reverse transcriptase inhibitor (NNRTI). For models, ART use was categorized using the following six categories: (1) ART-naïve; (2) no current ART but previous ART use; (3) current non-HAART ART; (4) HAART with no PI (*i.e.*, only NNRTI-based HAART); (5) current HAART containing any PI; and (6) current HAART containing the PI nelfinavir. Stata version 8.2 (College Station, TX) and SAS version 8.2 (SAS Institute) statistical software was used for all analyses.

3. Results

Study participants. 142 HIV-1 and HHV-8 co-infected men participated in the study; 115 (81%) reported their race as white (Table 1). The median time since HIV diagnosis was 10 years (range 0–24 years). The median CD4 count at first session was 385 cells/uL, with a wide range (1–1240). Of 132 participants with valid measures at first session, 61 (46%), 24 (18%) and 47 (36%) had a HIV VL of <50, 50–10,000, and >10,000 copies/mL, respectively.

Oropharyngeal swabs. The 142 participants collected a total of 11,608 oral swabs for HHV-8 DNA quantification (Table 2). A median of 40 daily swabs (range 14–129) was obtained during 262 sessions, and 74 (52%) participants collected more than one session over time for a total of 262 sessions. Among those with multiple sessions, the median time between sessions was 6 months (range <1 month–29 months).

Antiretroviral therapy use. A wide variety of ART use was observed among study participants (Table 2). While 128 participants (90%) either used the same combination of ART classes for all sessions or were not taking ART during all sessions,