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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.

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Competing Interests: None declared.

Ethical Approval: Written informed consent was obtained in accordance with a protocol approved by the University of Washington Human Subjects Division.

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 3,016 (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 to 0.5) compared to ART-naïve persons, whereas HAART without a PI was not (RR 0.7; 95% CI 0.4 to 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.

Keywords

Human herpesvirus 8; Kaposi sarcoma; antiretroviral therapy; protease inhibitor

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; 3 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 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.

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 CD4 T 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] CD4 T cell counts were measured with flow cytometry and HIV-1 plasma RNA was quantified using the AMPLICOR Monitor HIV-1 Test (Roche, Alameda, CA).

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 6 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, Texas) and SAS version 8.2 (SAS Institute) statistical software was used for all analyses.

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 to 1240). Of 132 participants with valid measures at first session, 61 (46%), 24 (18%) and 47 (36%) had a HIV VL of <50, 50 to 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 to 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, 14 participants changed ART regimen classifications between sessions and thus appear in multiple categories. Forty-six participants collected oropharyngeal swabs (3,111 (27%) of 11,608 total swabs) at a time when they were naïve to ART, 20 participants (1,272 (11%) of swabs) were not taking ART at the time of swab collection but had previously taken ART, 87 participants (6,564 (57%) of swabs) were taking HAART, and 9 (661 (6%) of swabs) were taking ART that did not meet the definition of HAART.

Of persons on HAART, 32 participants (2,216 (34%) of 6,564 swabs) were taking a NNRTI-based regimen without a PI, and 55 (4,348 (66%) of swabs) were taking a PI-based regimen, of whom 9 (587 (14%) of 4,348 swabs) were taking a regimen that included both a NNRTI and a PI. NRTI combinations included zidovudine/lamivudine, stavudine/lamivudine, didanosine/lamivudine and abacavir/lamivudine. The most commonly used PI was nelfinavir (1,139 (26%) of 4,348 swabs). Other PIs used were indinavir, saquinavir, ritonavir, ritonavir-boosted lopinavir, amprenavir, fosamprenavir, and atazanavir.

HHV-8 oropharyngeal shedding frequency and ART use

HHV-8 DNA was detected from the oropharynx in 3,016 (26%) of 11,608 swabs collected (Table 2). The frequency of HHV-8 shedding was 18.9% (1243 of 6,564 swabs) among participants using HAART and 39.6% in those who were ART-naïve. The lowest rates of shedding were observed persons taking PI-containing HAART (13.2%, or 572 of 4348 swabs). Participants receiving regimens specifically containing the PI nelfinavir also showed a relatively low rate of shedding (19.1%, or 217 of 1,139 swabs).

PI-based HAART was associated with a 80% lower rate of shedding (RR 0.2; 95% CI 0.1 to 0.5) in univariate analysis, whereas previous ART, non-HAART ART, or HAART without a PI did not significantly decrease shedding frequency compared to ART-naïve persons (Table 3). Nelfinavir-based HAART was not significantly associated with a reduction in HHV-8 shedding frequency (RR 0.7; 95% CI 0.2 to 1.9). HHV-8 shedding frequency was not significantly associated with low CD4 T cell count, high HIV VL, time since HIV diagnosis, or year of participation, but was greatly increased by the presence of KS (RR 2.5; 95% CI 1.5 to 4.1). The associations between ART regimens and HHV-8 shedding frequency were not markedly changed by adjusting for KS status in multivariate analysis (Table 3).

HHV-8 oropharyngeal shedding quantity and ART use

The mean quantity of HHV-8 detected among subjects receiving different ART regimens in shown in Table 2. Use of HAART with or without a PI showed a non-significant trend toward lower quantity with PI use (Table 4). Previous use of ART was associated with a mean decrease of 0.7 log copies (95% CI -1.3 to 0.0 log) and current non-HAART ART was associated with a mean increase of 0.3 log copies (95% CI 0.0 to 0.6 log) in the quantity of HHV-8 detected compared to ART-naïve persons in multivariate analysis. Compared to those participants without KS, the presence of KS was also associated with a mean increase of 0.9 log copies of HHV-8 (95% CI 0.2 to 1.6 log) in multivariate analysis. No clear trend was observed between HIV VL and the quantity of HHV-8 detected in oropharyngeal swabs. Those participants with a HIV VL between 500 and 10,000 copies/ml had a mean quantity of HHV-8 that was 0.6 log higher (95% CI 0.1 to 1.0 logs) compared to persons with HIV VL <500 copies/mL, while the mean quantity of HHV-8 among those with >100,000 copies of HIV/ml tended to be lower (coefficient -0.8; 95% CI -2.0 to 0.3 log) compared to those with HIV VL <50 copies/mL. Similarly, a direct association was observed between quantity of HHV-8 and CD4 count, in which the mean copy number was 0.4 log lower (95% CI -0.6 to -0.2 log) among those participants with a CD4 count <200, compared to those with a CD4 count ≥200. Neither time since HIV diagnosis nor the year of participation was associated with the HHV-8 quantity detected in the oropharynx.

Discussion

In our study that evaluated the effect of different ART regimens on HHV-8 shedding, in a cohort of 142 men with HIV-1 and HHV-8 co-infection who had daily sampling for quantitative HHV-8 PCR, on >11,000 days, PI-based HAART was associated with a significantly lower frequency of oral viral shedding compared to ART-naïve persons. In contrast, ART that did not contain a PI did not appear to decrease shedding frequency. A trend for reduced HHV-8 shedding quantity among PI-based HAART users was also observed. This study is limited by its observational design and inability to evaluate the effects of ART combinations and other risk factors in greater detail. For example, the duration of the ART regimens used and the level of medication adherence were unknown, but could affect HHV-8 shedding. A trend toward less frequent HHV-8 detection as well as significantly reduced HHV-8 quantity was observed among subjects with previous ART use but who were not on treatment during the study, but it is unknown what regimens they had received or when they were discontinued. Choice of drug regimen is influenced by clinical factors, potentially leading to confounding by indication, where the observed relationship between HHV-8 shedding patterns and antiretroviral use is related not to the use of a specific ART regimen but rather to its indication.[45] For example, if clinicians prescribed PI-based therapy to individuals with a common characteristic (previous resistance to NNRTIs, significant comorbidities, etc.), then the observed relationship between PI use and lower HHV-8 shedding could instead be attributable to the factor influencing the choice of ART. We were also limited in our ability to evaluate the effect of any individual antiretroviral agents on HHV-8 shedding due to the large number of drug combinations used. Finally, although HHV-8 shedding reflects viral replication in the oropharynx and appears to occur prior to systemic replication,[40, 46] our study did not have the ability to evaluate other measures of HHV-8 replication such as viremia.

Prevention of KS is an important goal given that KS resolution rates range between only 44%-60% despite HAART and chemotherapy treatment.[5-9] The optimal strategy to prevent KS is not clear, but there is evidence that suppression of HHV-8 replication may be highly advantageous.[30, 47] Remarkably, *in vitro* studies have shown that some PIs decrease inflammatory cytokine production implicated in HHV-8 reactivation[48, 49] and one, nelfinavir, inhibits HHV-8 replication directly.[34] An inhibitory effect of nelfinavir on HHV-8 shedding was not observed in this cohort. While this may indicate that nelfinavir is inactive against HHV-8 replication *in vivo*, it is also possible that small numbers and the presence of residual confounding in the analyses obscured finding an effect. Alternatively, measurement of HHV-8 DNA alone may not accurately reflect the antiviral activity of nelfinavir; although the drug blocks generation of infectious HHV-8 virions *in vitro*, it appears to act late in virus production after DNA replication and may actually induce reactivation of latent virus.[50] These findings together with the *in vivo* results reported here lend additional support to the hypothesis that PIs may have anti-HHV-8 activities that impede progression to KS. On the other hand, observational cohort studies of KS development in Western countries have reported similar risk reduction with PI- and NNRTI-based HAART.[27, 51] This may be explained in part by the varying anti-tumor and anti-

HHV-8 activities of different PIs,[32, 48, 49] such that when lumped together the beneficial effects of some agents could be obscured.

Whether PI-based HAART should be preferentially used for treatment of KS is a matter of debate. Observational studies of the effect of HAART type on KS response have yielded variable conclusions.[7, 24, 27, 28, 49, 52-54] One uncontrolled trial of indinavir suggested a clinical benefit for classic (HIV-negative) KS.[55] Indinavir, ritonavir, lopinavir and other PIs have anti- angiogenic and anti-tumor properties, though they appear to be less potent than nelfinavir and lack anti-HHV-8 activity *in vitro*.[33, 34] As such, KS treatment and prevention trials should be considered to specifically evaluate nelfinavir or other agents with inhibitory activity against HHV-8.[30, 47]

In summary, our data suggest that PI-based HAART may suppress HHV-8 infection more effectively than regimens without PIs, and may therefore confer particular benefits for individuals at high-risk for KS development and disease progression. Controlled trials are needed to definitively determine whether specific PI-based regimens differentially suppress HHV-8 oropharyngeal replication or have other beneficial effects for the prevention and treatment of KS, particularly in areas with a high burden of KS.

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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)
CD4 T cell count (cells/|al), 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)

Table 2

Antiretroviral therapy use and frequency of HHV-8 detected by PCR of oropharyngeal swabs

Antiretroviral use	Subjects (n)	Sessions (n)	Proportion of swabs with HHV-8 detected (%)	Mean $\log_{10}$ copies (SD)
ART-naïve	46	68	1231/ 3111 (39.6%)	4.9 (1.2)
Previous ART only	20	28	314/ 1272 (24.7%)	4.1 (1.0)
Non-HAART ART	9	12	228 / 661 (34.5%)	4.5 (1.1)
HAART, no PI	32	56	671 / 2216 (30.3%)	4.9 (1.2)
HAART with any PI	55	98	572 / 4348 (13.2%)	4.4 (1.1)
HAART with nelfinavir	12	21	217 / 1139 (19.1%)	4.9 (1.1)
Total	162	262	3,016 / 11,608 (26.0%)	4.7 (1.2)

*Among sessions with ≥ 1 positive day

Abbreviations: HHV-8, human herpesvirus 8; ART, antiretroviral therapy; HAART, highly active ART; PI, protease inhibitor; SD, standard deviation

Table 3

Associations between variables and frequency of HHV-8 detection in oropharyngeal swabs by PCR.

	Univariate		Multivariate	
Covariate	**RR (95% CI)**	**p**	**RR (95% CI)**	**p**
Antiretroviral use				
ART-naïve	ref		ref	
Previous ARVs only	0.6 (0.3, 1.1)	0.117	0.6 (0.3, 1.0)	0.061
Current ART, not HAART	0.9 (0.5, 1.7)	0.781	0.8 (0.3, 2.0)	0.593
HAART, no PI	0.7 (0.4, 1.3)	0.279	0.6 (0.3, 1.1)	0.088
HAART with any PI	0.2 (0.1, 0.5)	0.002	0.2 (0.1, 0.4)	<0.001
HAART with nelfinavir	0.7 (0.2, 1.9)	0.465	0.6 (0.2, 1.7)	0.361
CD4 count				
CD4 >= 200	ref			
CD4 < 200	0.9 (0.5, 1.5)	0.663		
Kaposi sarcoma				
KS negative	ref		ref	
KS positive	2.5 (1.5, 4.1)	0.001	3.5 (1.8, 6.9)	<0.001
HIV plasma viral load				
<500 copies/mL	ref			
500 to 10K copies/mL	1.9 (1.1, 3.1)	.017		
10K to 100K copies/mL	1.3 (0.6, 2.7)	.513		
>100K copies/mL	1.0 (0.6, 1.7)	.943		
Decades since HIV dx	0.6 (0.4, 1.0)	.077		
Year of participation	1.0 (0.9, 1.0)	.260		

Multivariate models are adjusted for all of the variables listed.

Table 4

Associations between variables and quantity of HHV-8 detected in oropharyngeal swabs by PCR.

	Univariate		Multivariate	
Covariate	**Log_{10} change (95% CI)**	**p**	**Log_{10} change (95% CI)**	**p**
Antiretroviral use				
ART-naïve	ref		ref	
Previous ARVs only	-0.6 (-1.3, 0.0)	0.06	-0.7 (-1.3, 0.0)	0.039
ART, not HAART	0.5 (0.2, 0.8)	0.001	0.3 (0.0, 0.6)	0.022
HAART, no PI	0.1 (-1.4, 1.7)	0.88	0.1 (-1.5, 1.6)	0.93
HAART with any PI	-0.7 (-1.5, 0.1)	0.098	-0.8 (-1.7, 0.0)	0.052
HAART with nelfinavir	0.4 (-0.5, 1.4)	0.37	0.0 (-1.0, 1.0)	0.95
CD4 count				
CD4 >= 200	ref		ref	
CD4 < 200	-0.6 (-0.8, -0.3)	<0.001	-0.4 (-0.6, -0.2)	<0.001
Kaposi sarcoma				
KS negative	ref		ref	
KS positive	0.4 (-0.3, 1.0)	.28	0.9 (0.2, 1.6)	0.016
HIV plasma viral load				
<500 copies/mL	ref			
500 to 10K copies/mL	0.6 (0.1, 1.0)	.012		
10K to 100K copies/mL	0.4 (-0.6, 0.6)	.90		
>100K copies/mL	-0.8 (-2.0, 0.3)	.16		
Decades since HIV dx	0.1 (-1.0, 1.1)	.88		
Year of participation	0.0 (-0.1, 0.2)	.58		

Multivariate models are adjusted for all of the variables listed.