AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 25, Number 2, 2009

DOI: 10.1089/aid.2008.0196

Recreational Drug Use and Risk of Kaposi's Sarcoma in HIV- and HHV-8-Coinfected Homosexual Men

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Abstract

Experimental data suggested that exposure to recreational drugs might adversely affect antitumor immunity, which led us to examine the hypothesis that use of marijuana, cocaine, poppers, and amphetamines might increase the risk of Kaposi's Sarcoma (KS) in HIV- and HHV-8-coinfected homosexual men. We analyzed data prospectively collected from the Multicenter AIDS Cohort Study (MACS) between 1984 and 2002. Among the 1335 HIV- and HHV-8-coinfected white men, 401 KS cases were identified. Multivariable Cox regression models were used to estimate the effects of time-varying recreational drug use on KS risk adjusting for potential confounders. The effects of both recent use (6 months prior) of recreational drugs and lagged exposure (i.e., use from 3 and 5 years prior) were examined. We did not observe any clear association with KS for recent use of any of the four drugs. In the analyses using lagged exposures, KS risk was associated with use of poppers 3–5 years prior [hazard ratio $(HR)_{3\ \text{years prior}} = 1.27$, 95% CI (0.97–1.67), $HR_{5\ \text{years prior}} = 1.46$ (1.01–2.13)]. However, no clear dose–response relationship was observed. These findings do not support a biological association between use of these substances and KS development in HIV- and HHV-8-coinfected homosexual men.

Introduction

The incidence of Kaposi's Sarcoma (KS) in the United States has largely declined after the introduction of highly active antiretroviral therapy (HAART).[1,2] However, KS still is a significant cause of morbidity and mortality in persons with HIV/AIDS.[3] Most HIV-related KS in the United States has been diagnosed in men who have sex with men (MSM).[4–6] Infection with a sexually transmitted agent, human herpesvirus 8 (HHV-8, also known as Kaposi's sarcoma-associated herpesvirus, KSHV) is now the only factor known to be essential for KS development.[3,7] Although HHV-8 infection is necessary, it is not a sufficient cause for the disease. Martin *et al.*[8] reported a 10-year KS risk of 50% among men who were coinfected with HIV and HHV-8 in the pre-HAART era. It is therefore possible that other factors may enhance the oncogenic potential of HHV-8 in HIV-infected persons.

Research based on *in vitro* and animal models, as well as in humans, has shown that the most commonly used recreational drugs in MSM, such as marijuana, cocaine, poppers, and amphetamines, have immunomodulatory properties. Results from several experimental studies demonstrated that marijuana affects the function of T and B lymphocytes, natural killer (NK) cells, and macrophages as well as the cytokine network.[9–15] Some studies suggested that marijuana might specifically inhibit antitumor immunity and accelerate tumor growth.[16–19] Other studies have suggested that exposure to cocaine is associated with alterations in activation and/or function of various lymphocytes and immune effector cells, and may lead to reduced cell-mediated immune function

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favoring tumor formation and infection.[20–22] The use of poppers has also been shown to result in immunosuppression, especially in human NK cell activity,[23] and to facilitate tumor growth in mice by suppressing immune function.[24,25] Amphetamines have been shown to suppress T cell and B cell function and proliferation in mouse models and *in vitro*.[26,27] These findings led to the hypothesis that use of these substances may increase the risk of developing KS in HIV- and HHV-8-coinfected individuals by suppressing ant-tumor immunity. Other animal and *in vitro* studies have also suggested the possibility that use of marijuana and cocaine may alter the risk of HIV-related KS by enhancing HIV replication[28,29] and/or by interacting with the course of HHV-8 infection.[30,31]

Few epidemiologic studies have examined the effect of substance use on the risk of developing HIV-related KS. One study examined risk factors for KS among HHV-8-seropositive men with AIDS and found that tobacco smoking and cocaine use were associated with lower KS prevalence.[32] An earlier analysis based on the Multicenter AIDS Cohort Study (MACS)[33] between 1984 and 1992 employing a nested case–control design found that use of poppers was associated with KS. However, this study did not account for HHV-8 infection status. In the current study, which includes more KS cases than previously studied, we examined the hypothesis that use of marijuana, cocaine, poppers, and amphetamines may increase the risk of developing KS in HIV- and HHV-8-coinfected men. We conducted survival analyses using the Cox proportional hazards model, taking advantage of the prospective nature of the study with repeated exposure measurements.

Materials and Methods

Study population

The MACS, started in 1984, is an ongoing cohort study of the natural and treated histories of HIV-1 infection in MSM.[34,35] Data collection centers are located in Chicago, IL, Baltimore, MD/Washington, DC, Pittsburgh, PA, and Los Angeles, CA. The study had three recruitment periods: (1) April 1984 to March 1985, when 4954 predominately white men were enrolled; (2) April 1987 to September 1991, when 668 ethnically more diverse men were enrolled; and (3) October 2001 to August 2003, when 1350 men, primarily African-American and Latino, were enrolled. Our study was based on men in the first and second recruitment periods (referred to as the pre-2001 cohort in the following text) followed to the end of year 2002. Every 6 months, the men in the MACS complete an interviewer-administered questionnaire and a physical examination. The interview requests information on demographic characteristics, behaviors (e.g., sexual practices, recreational drug use, tobacco smoking, alcohol drinking), and medical history (e.g., AIDS-related symptoms and medications prescribed). Blood is collected at each visit for virologic, serologic, immunologic, and other laboratory measurements, and for a repository of serum, plasma, and peripheral blood mononuclear cells.

Outcome

Malignancy outcomes are continuously monitored in MACS. Medical records are obtained and reviewed to confirm reports of malignancies. We identified specific malignancies defined by the International Classification for Disease-Oncology version 3 (ICDO-3) topology and morphology codes, available in the MACS dataset. KS was identified by morphology code 9140.3 for this analysis.

HHV-8 serostatus

HHV-8 serostatus was measured in 2127 men in the MACS pre-2001 cohort. Several groups of participants have been sampled for HHV-8 testing in the MACS for various research purposes. These groups include (1) HIV seroconverters who had a less than 1 year window between their last HIV-seronegative and their first HIV-seropositive visit and whose serum sample was available; (2) a random sample of 400 HIV-seropositive and 100 HIV-seronegative participants at time of study enrollment; (3) HIV-seropositive participants at visits 3 or 4 who had CD4 cell count and HIV viral load measurement at the visit; (4) most of the cohort participants at one MACS site (Pittsburgh, PA); (5) most of the KS cases; and (6) participants in two nested case–control studies.

HHV-8 serostatus was determined from serum samples stored at -70°C. An indirect immunofluorescence assay was used to detect antibodies directed against replicative (lytic) HHV-8 viral proteins with the BCBL-1 cell line (NIH AIDS Research Reagent Program, Rockville, MD).[36]

Variables

Use of marijuana, cocaine, poppers, and amphetamines was first modeled as binary variables (use vs. no use since last visit). To further assess if there was a threshold effect and to examine the dose–response relationship, substance use was further modeled as frequency of use: no use (reference), monthly or less frequent use, and weekly or more frequent use. A test for trend was conducted by treating substance use as a continuous variable with the following coding: 0 if no use, 12 if monthly or less frequent use, and 52 if weekly or more frequent use. Use of amphetamines was not specifically asked between visits 16 and 21 (October 1991–September 1994). Therefore, amphetamine use status at these visits was assumed to be the same as that reported at visit 15. The following potential confounders were adjusted for in every model: age, college education, study center, alcohol use (no use, $\leq$2 times/week, $>$2 times/week), tobacco smoking (no, $<$1 pack/day, 1 $\sim$ $<$2 pack/day, and $\geq$2 packs/day), number of male sexual partners since the last study visit ($<$6 partners and $\geq$6 partners), lifetime number of sexual partners (at the time of study enrollment, quartiles), receptive anal intercourse (RA) and condom use (no RA, RA with condom use at all times, and RA without consistent condom use), antiretroviral therapy (single, combination, and HAART), CD4 cell count (continuous), and sexually transmitted infection score (scores were based on one point for each of the following conditions that were shown to be associated with the risk of KS in MACS:[33] hepatitis, gonorrhea, herpes, genital/anal warts, and scabies; scores ranged from 0 to 5). We used the CD4 cell count obtained from the previous visit to control for the potential confounding effect of this variable on substance use. This ensured that the CD4 cell count included in the models preceded the substance use reported in the current visit. Use of the four substances was simultaneously included in the models.

Statistical analysis

The primary objective of this study was to examine the associations between substance use and risk of KS among HIV- and HHV-8-coinfected individuals. Therefore, our study was based on persons who were HIV seropositive at the time of enrollment (seroprevalent) and those who subsequently acquired HIV (seroconverted). The analyses were further restricted to HHV-8-positive person-time among those who were tested for HHV-8. Follow-up started at the first HIV- and HHV-8-coinfected visit in the study period and ended at either the time of KS diagnosis or the last KS-free visit in the study period. For persons who were diagnosed with KS at the time of death, the time of KS development was estimated as the mid-point between the time of the last study visit and the time of death. Two cases for which the time of diagnosis of KS was unavailable were excluded from the analysis. Since the majority (93%) of the KS cases involved men of the white race, we restricted our analysis to white men.

To understand the relationship between substance use and potential confounding factors, we used univariate logistic regression to examine the crude associations between recreational drug use (yes or no, dependent variable) and potential confounding factors at the time of study enrollment. We examined the effects of time-varying exposures to substances by fitting a time-dependent Cox regression model.[37] To ensure that the reported behaviors and immunologic measures preceded the diagnosis of the disease, we employed a one visit lag (approximately 6-month lag) in our analyses. To further explore the effect of the four substances in the most relevant exposure period, we conducted lagged analyses employing different assumptions for the average induction time of HIV-related KS. We conducted two sets of analyses, involving the assumption for average induction time of 3 and 5 years. In the 3 years lagged analysis, we used the exposure status for all covariates from at least 3 previous years prior to diagnosis, and similarly for the 5 years lagged analysis. Exposure was lagged for the assumed relevant exposure period for all men in the analyses, regardless of their KS status.

Since use of one substance was expected to be correlated with use of other substances,[38,39] the following informal diagnostic methods were used to assess the presence of multicolinearity:[40] (1) examination of the statistical associations between these variables; (2) removing use of marijuana, cocaine, poppers, and amphetamine variables one at a time to see if large changes (e.g., >20%) in the estimated regression coefficients resulted; (3) modeling use of one substance at a time without adjusting for use of the other three drugs of interest; and (4) examination of whether there was a nonsignificant coefficient for an important predictor, such as CD4 cell count.

Due to the lack of regular longitudinal testing for HHV-8 and inconsistent test results, a number of KS cases (20%) were not included in the primary analysis for HIV- and HHV-8-coinfected men. Since these KS cases were most likely HHV-8 infected, we also conducted a sensitivity analysis including all identified KS cases. A proportional hazards assumption was examined by testing the significance of interaction terms between the four drugs of interest and categorical variables for follow-up time periods. All analyses were conducted using SAS statistical software version 8 (Statistical Analyses System Inc., Cary, NC).

Results

A total of 2237 men were HIV positive at enrollment, and an additional 522 men HIV seroconverted before 2003. After applying the exclusion criteria, 1335 white men were included in the study, providing a total of 10,423 HIV- and HHV-8-coinfected person-years. We identified 500 KS cases diagnosed in the study period (95% diagnosed in the pre-HAART era). Among them, 401 (80%) were diagnosed while they were confirmed HHV-8 positive. HHV-8 status was indeterminate for 95 KS cases, and four men were diagnosed with KS despite being HHV-8 negative (these were likely false-negative results). The indeterminate HHV-8 status for the 95 KS cases was due to lack of HHV-8 testing between the initial HHV-8-seronegative visit and the time of KS diagnosis (52% of the indeterminate cases), or a negative testing results after the initial HHV-8-seropositive visit (48% of the indeterminate cases). These 99 KS cases were therefore excluded from the primary analysis, but were included in the sensitivity analysis. The HIV- and HHV-8-positive cohort included mostly middle-aged men with at least some college education. Table 1 shows the demographic, recreational drug us, and other behavioral risk factors of the 1335 white men.

Use of recreational drugs was associated with demographic and behavioral risk factors (Table 2). Younger men tended to use marijuana, cocaine, and amphetamines. Use of cocaine and amphetamines was also associated with lower education. Use of all four substances was positively associated with tobacco smoking and alcohol drinking, as well as higher numbers of sexual partners and a history of more sexually transmitted infections. Substance use appeared to be associated with risky sexual practices, i.e., inconsistent or no use of condoms. Use of any of the four substances was also associated with use of the other three. Use of poppers was, however, less strongly associated with use of other substances than use of marijuana, cocaine, and amphetamines.

In the univariate analyses among HIV- and HHV-8-coinfected men, frequent use of cocaine (at least weekly) and poppers (at least weekly) was inversely associated with KS risk [Table 3, crude hazard ratio (HR)]. In the multivariable analyses, we did not observe a clear association between use of marijuana, poppers, or amphetamines and KS risk (Table 3, recent use). Weekly or more frequent use of cocaine was inversely associated with KS risk [HR = 0.38, 95% CI (0.14–1.04)]. In the lagged analyses, a positive association with KS risk was found for use of poppers 3 and 5 years prior to KS diagnosis [$HR_{3\ \text{years prior use}} = 1.27$ (0.97–1.67), $HR_{5\ \text{years prior use}} = 1.46$ (1.04–2.06), Table 3]. However, no clear dose–response relationship was observed, as the HR estimates were of similar magnitude for monthly or less frequent use and for weekly or more frequent use. In the 5 years lagged analysis, a positive association was observed for weekly or more frequent use of marijuana [HR = 1.52 (0.99–2.32)]. No clear associations were found for 3–5 years prior use of cocaine or amphetamines. In the sensitivity analyses including all KS cases, the inverse association was no longer found for recent frequent use of cocaine. The association between 5 years prior frequent use of marijuana and KS was also no longer significant [HR = 1.33 (0.94–1.89)].

We also found that among HIV- and HHV-8-coinfected men, older men with higher CD4 cell counts were less likely to

Table 1. Demographic, Substance Use, Sexual Behaviors, and CD4 Cell Count in HIV and HHV-8-Coinfected White Men

Characteristics at first HIV and HHV-8-positive visit	Number (%)[a]	
	Subjects (N = 1335)	Person-years (n = 10,423)
Age (years)	33.8 (29.7/38.6)[b]	
Education		
Less then college degree	615 (46.1)	
College degree or higher	709 (53.1)	
Use of marijuana in the past 6 months		
None	377 (28.2)	4777 (45.8)
Monthly or less frequent	516 (38.7)	3145 (30.2)
Weekly or more frequent	437 (32.7)	2427 (23.3)
Use of cocaine in the past 6 months		
None	822 (61.6)	8190 (78.6)
Monthly or less frequent	460 (34.5)	1889 (18.1)
Weekly or more frequent	50 (3.8)	275 (2.6)
Use of amyl nitrites in the past 6 months		
None	442 (33.1)	5884 (56.5)
Monthly or less frequent	536 (40.2)	2879 (27.6)
Weekly	351 (26.3)	1579 (15.1)
Use of amphetamines in the past 6 months		
None	995 (74.5)	8760 (84.0)
Monthly or less frequent	270 (20.2)	1019 (9.8)
Weekly or more frequent	43 (3.2)	204 (2.0)
Tobacco smoking in the past 6 months		
No	846 (63.4)	7107 (68.2)
Yes	486 (36.4)	3294 (31.6)
Alcohol drinking in the past 6 months		
No	119 (8.9)	1672 (16.0)
≤2 times/week	665 (49.8)	5425 (52.0)
>2 times/week	540 (40.5)	3305 (31.7)
Number of male sexual partners in the past 6 months		
<6	770 (57.7)	7674 (73.6)
≥6	563 (42.2)	2594 (24.9)
Number of lifetime sexual partners[c]	310 (120/999)[b]	
Receptive anal sex (RA) in the past 6 months		
No	331 (24.8)	4783 (45.9)
RA with use of condoms at all times	174 (13.0)	2585 (24.8)
RA with inconsistent or no use of condom	816 (61.1)	2843 (27.3)
Sexually transmitted infection score		
0	67 (5.0)	388 (3.7)
1–2	604 (44.2)	3838 (36.8)
3–5	651 (48.8)	6113 (58.6)
Baseline CD4 lymphocyte count (/μl)	554 (381/739)[b]	

[a]Percent may not add up to 100% due to missing values.
[b]Median (25/75 percentile).
[c]Number of lifetime sexual partners at time of study enrollment.

develop KS. Antiretroviral therapy, particularly HAART, was associated with significantly reduced KS risk. In the lagged analyses, 3–5 years prior receptive anal sex without use of a condom was independently associated with increased KS risk [$HR_{3\ years\ prior} = 1.50$ (1.10–2.05), $HR_{5\ years\ prior} = 2.02$ (1.39–2.95)]. We did not find any association between KS risk and tobacco smoking.

The estimates for regression coefficients and variances for use of any of the four substances were not sensitive to the inclusion or exclusion of use of the other three drugs. We also did not find any hazard ratio in the opposite direction to that expected, nor did important predictors such as CD4 cell count or HAART use become nonsignificant, suggesting that multicolinearity was unlikely to have significantly affected the validity of our results. We did not find violations for the proportional hazards assumption in these models.

Discussion

We did not find a clear association between use of any of the four substances and risk of KS in HIV- and HHV-8-coinfected white men. In the lagged analyses, 3–5 years prior use of poppers was associated with KS. However, a clear dose–response relationship was lacking. These observations did not support our hypothesis that use of marijuana, cocaine, poppers, and amphetamines adversely affects KS risk by further

Table 2. Univariate Associations between Recreational Drug Use and Demographic, Behavioral Risk Factors, and CD4 Cell Count[a]

	OR (95 % CI)			
	Marijuana use	*Cocaine use*	*Poppers use*	*Amphetamine use*
Age (per 10 years increase)	0.61 (0.52–0.73)	0.57 (0.48–0.68)	0.87 (0.74–1.03)	0.45 (0.36–0.56)
Education				
Less then college degree	1	1	1	1
College degree or higher	0.87 (0.68–1.11)	0.72 (0.58–0.90)	1.06 (0.84–1.33)	0.52 (0.40–0.67)
Use of marijuana in the past 6 months				
No	—	1	1	1
Yes	—	8.45 (5.93–12.05)	4.31 (3.35–5.55)	7.14 (5.45–11.23)
Use of cocaine in the past 6 months				
No	—	—	1	1
Yes	—	—	2.48 (1.93–3.19)	4.70 (3.59–6.16)
Use of poppers in the past 6 months				
No	—	—	—	1
Yes	—	—	—	3.71 (2.65–5.20)
Tobacco smoking in the past 6 months				
No	1	1	1	1
Yes	2.27 (1.73–2.97)	1.48 (1.18–1.86)	1.48 (1.16–1.88)	1.84 (1.42–2.38)
Alcohol drinking in the past 6 months				
No	1	1	1	1
≤2 times/week	4.64 (3.07–7.01)	3.55 (2.02–6.25)	3.48 (2.31–5.23)	2.25 (1.20–4.22)
>2 times/week	7.32 (4.75–11.26)	6.68 (3.79–11.77)	5.70 (3.73–8.71)	3.77 (2.02–7.05)
Number of male sexual partners in the past 6 months				
<6	1	1	1	1
≥6	1.52 (1.19–1.94)	1.51 (1.21–1.89)	4.12 (3.16–5.36)	1.67 (1.30–2.16)
Risky sexual practice in the past 6 months				
No receptive anal intercourse (RA)	1	1	1	1
RA with use of condoms at all times	1.47 (1.00–2.16)	0.94 (0.63–1.40)	1.77 (1.22–2.57)	1.14 (0.69–1.86)
RA with inconsistent or no use of condom	2.51 (1.90–3.29)	1.59 (1.22–2.09)	3.49 (2.67–4.57)	2.04 (1.47–2.84)
History of sexually transmitted infections				
0	1	1	1	1
1–2	1.30 (0.76–2.20)	1.78 (1.00–3.16)	1.54 (0.92–2.56)	1.44 (0.75–2.76)
3–5	1.59 (0.94–2.70)	1.99 (1.12–3.53)	1.82 (1.10–3.04)	1.40 (0.73–2.69)
CD4 lymphocyte count (per 100/μl increase)	1.04 (0.99–1.08)	1.02 (0.98–1.06)	1.05 (1.01–1.09)	1.02 (0.98–1.07)

[a]Among the 1335 white men at the first HIV and HHV-8-positive visit.

suppressing the immune system in HIV- and HHV-8-coinfected men.

Although the lack of dose–response did not support our hypothesis, the modest association found for poppers in the lagged analyses may suggest that use of poppers, even occasionally, may be a marker for acquiring additional KS risk factors among MSM coinfected with HIV and HHV-8. Use of poppers has been consistently found to be associated with HHV-8 seropositivity.[41–43] Although our analyses were restricted to HHV-8-seropositive men and adjusted for sexual risk factors, use of poppers might still be associated with unsafe sexual behaviors[44,45] and hence repeated exposure to HHV-8 and a higher HHV-8 infection load. The hypothesis that use of poppers may be a marker for other risk factors for KS among HHV-8-infected men should be studied further, including risk factors not included in this study. Future research should examine the relationship between use of poppers, repeated exposure to unprotected sex, HHV-8 viral load, and KS risk.

We observed some discrepancies between the results for recent drug use and drug use from 3–5 years prior. Specifically, there was no association for recent poppers use and an inverse association for recent cocaine use. It is possible that the decline of the immune function and/or cancer symptoms may affect poppers or cocaine use behaviors in these men prior to KS diagnosis. Therefore, a possible temporal ambiguity should be considered when interpreting the results for recent substance use.

Forty-six KS cases had an indeterminate HHV-8 serostatus due to a negative test result after an initial HHV-8 positive test (these cases were not included in the primary analyses but were included in the sensitivity analyses). This is likely false-negative test results. However, the lytic immunofluorescence assay is considered one of the most sensitive assays available for HHV-8 antibody testing,[46–48] with an estimated sensitivity of up to 95%.[47] Furthermore, we have also retested the HHV-8 serostatus for the majority of these KS cases and obtained consistent seronegative results. Therefore, alternatively, this could indicate the inability to generate antibody when the men were severely immunocompromised.[49,50] The mean and median CD4 cell count of these men when they were tested HHV-8 seronegative was 212 cells/μl and 119 cells/μl, respecespectively. Netski *et al.*[49] reported that CD4 cell count <500 cells/μl was significantly associated with lower antibody

Table 3. Multivariable Cox Proportional Hazards Models on Time to KS: HIV- and HHV-8-Coinfected Cohort

		Recent use (6 months prior)			Exploratory Lagged Analyses			
					Three years prior use		Five years prior use	
	KS (no.)	Crude HR (95% CI)	HR[a] (95% CI)	p-trend	HR[a] (95% CI)	p-trend	HR[a] (95% CI)	p-trend
Use of marijuana		0.95 (0.78–1.16)[b]	1.00 (0.79–1.28)[b]		1.05 (0.79–1.39)[b]		1.25 (0.87–1.79)[b]	
None	187	1	1		1		1	
Monthly or less frequent	120	0.96 (0.76–1.20)	0.99 (0.76–1.30)	0.97	1.01 (0.74–1.37)	0.35	1.15 (0.77–1.70)	0.71
Weekly or more frequent	85	0.94 (0.73–1.22)	0.98 (0.72–1.34)		1.13 (0.81–1.60)		1.52 (0.99–2.32)	
Use of cocaine		0.77 (0.59–1.00)[b]	0.85 (0.62–1.16)[b]		0.94 (0.70–1.27)[b]		1.24 (0.88–1.75)[b]	
None	325	1	1		1		1	
Monthly or less frequent	63	0.82 (0.62–1.08)	0.95 (0.69–1.32)	0.08	0.91 (0.67–1.25)	0.80	1.19 (0.83–1.71)	0.71
Weekly or more frequent	5	0.44 (0.18–1.07)	0.38 (0.14–1.04)		0.99 (0.53–1.84)		1.05 (0.50–2.20)	
Use of poppers		0.75 (0.61–0.92)[b]	1.14 (0.88–1.48)[b]		1.27 (0.97–1.67)[b]		1.46 (1.04–2.06)[b]	
None	254	1	1		1		1	
Monthly or less frequent	110	0.84 (0.66–1.06)	1.18 (0.90–1.56)	0.80	1.25 (0.93–1.69)	0.89	1.47 (1.01–2.13)	0.54
Weekly or more frequent	37	0.56 (0.40–0.79)	0.94 (0.64–1.40)		1.32 (0.93–1.87)		1.44 (0.94–2.20)	
Use of amphetamines		0.99 (0.74–1.34)[b]	1.28 (0.90–1.83)[b]		1.16 (0.83–1.62)[b]		0.85 (0.57–1.28)[b]	
None	330	1	1		1		1	
Monthly or less frequent	39	0.94 (0.68–1.32)	1.21 (0.83–1.78)	0.05	1.16 (0.81–1.66)	0.47	0.75 (0.47–1.18)	0.11
Weekly or more frequent	10	1.22 (0.65–2.30)	1.77 (0.88–3.56)		1.10 (0.53–2.29)		1.62 (0.78–3.36)	

[a]Estimates controlled for age, education, study center, tobacco smoking, alcohol use, number of male sexual partners since last visit, number of lifetime sexual partners at time of study enrollment, receptive anal intercourse and condom use, history of sexually transmitted infections, antiviral therapy, and CD4 cell counts.

[b]For binary substance use (yes vs. no). Estimates are derived from a model containing use of marijuana, cocaine, poppers, and amphetamines as binary variables.

titer to hepatitis C antigens. The effect of CD4 cell level on mounting and maintaining appropriate hormonal response in HIV-infected persons is not well known and should be further investigated.

One limitation of our study is the lack of consistent testing for HHV-8 for all participants in the pre-2001 MACS cohort and for determining the precise time point for HHV-8 seroconversion. This led to the exclusion of 95 men who developed KS at a time when the information of their HHV-8 serostatus was not available. However, the sampling methods for HHV-8 testing in MACS are unlikely to be associated with substance use. Furthermore, including these men in the sensitivity analysis did not affect our conclusions. Another limitation is that frequency of drug use was collected by prespecified categories (i.e., less than monthly, monthly, weekly, etc.), and these categories are not strictly continuous. As a result, there might be ambiguity in reporting levels of substance use (e.g., three times per month could be reported as monthly use or weekly use) and hampered our ability to examine the dose–response relationship.

Despite these limitations, our study has substantial strengths including a longitudinal design and measurement of variables at approximately 6-month intervals, allowing us to examine the effect of substance use from different exposure periods adjusting for multiple potential confounders. Our study adds to the limited knowledge of the clinical significance of recreational drug use and the etiology of KS. The results of this study do not support a biological adverse effect of use of the four substances on KS development in men coinfected with HIV and HHV-8.

Acknowledgments

The Multicenter AIDS Cohort Study includes the following: Baltimore, Maryland: The Johns Hopkins University Bloomberg School of Public Health (Principal Investigator: Joseph B. Margolick); Chicago, Illinois: Howard Brown Health Center, The Feinberg School of Medicine, Northwestern University, and Cook County Bureau of Health Services (Principal Investigator: John P. Phair, Co-Principal Investigator: Joan S. Chmiel); Los Angeles, California: University of California, UCLA Schools of Public Health and Medicine (Principal Investigator: Roger Detels, Co-Principal Investigator: Barbara R. Visscher); Pittsburgh, Pennsylvania: University of Pittsburgh, Graduate School of Public Health (Principal Investigator: Charles R. Rinaldo, Co-Principal Investigator: Lawrence Kingsley); Data Coordinating Center: The Johns Hopkins University Bloomberg School of Public Health (Principal Investigator: Lisa P. Jacobson, Co-Principal Investigator: Alvaro Muñoz); Bethesda, Maryland: National Institutes of Health, National Institute of Allergy and Infectious Diseases, and National Cancer Institute. This work is supported by the National Institute on Drug Abuse (NIDA)/NIH Grants RO1 DA8254 and R37 DA03018.

Disclosure Statement

No competing financial interests exist.

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