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Effect of Prophylactic Valacyclovir on the Presence of Human Herpesvirus DNA in Saliva of Healthy Individuals after Dental Treatment

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Human herpesviruses (HHVs) are ubiquitous pathogens that intermittently reactivate from latency. Transmission is believed to be facilitated by their frequent appearance in saliva. This study sought to understand the factors that influence the appearance of these viruses in saliva by examining the prevalence, pattern, and quantity of all eight HHVs in saliva of immunocompetent adults with a history of recurrent oral herpes simplex virus (HSV) infections following dental treatment and antiviral therapy. Valacyclovir or matched placebo was given (2 g twice on the day of treatment and 1 g twice the following day) to 125 patients in a randomized, double-blind controlled trial. Saliva, collected on the day of dental treatment and 3 and 7 days later, was analyzed using real-time quantitative PCR. At all visits, HHVs coinfected saliva. Over the course of the week, the DNAs of HHV-6 and HHV-7 were detected significantly more often (97% to 99% of patients) than Epstein-Barr virus (EBV; 64.8%), HSV-1 (13.0%), HHV-8 (3.2%), cytomegalovirus (2.4%), HSV-2 (0%), and varicella-zoster virus (0%), irrespective of drug treatment ($P < 0.002$). Mean genome copy numbers were highest for HSV-1 and HHV-6. Dental treatment did not influence asymptomatic viral shedding patterns. However, valacyclovir treatment resulted in significantly fewer patients shedding EBV at both postoperative visits compared with placebo ($P < 0.008$). These results suggest that HHVs are simultaneously present in the saliva of healthy adults at levels that could facilitate transmission, and valacyclovir therapy decreases the prevalence of EBV in saliva but has little effect on HHV-6 and HHV-7.

Herpesviruses are ubiquitous pathogens that infect many animal species, including humans. Eight distinct members of the human *Herpesviridae* family have been identified. They include herpes simplex virus type 1 (HSV-1), HSV-2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), HHV-7, and HHV-8 (Kaposi's sarcoma herpesvirus [KSHV]) (62, 81). These large DNA viruses each express more than 70 genes that specify a number of proteins and enzymes that can cause destruction of infected cells during the lytic infection (12, 63). Following the initial lytic infection, HHVs establish a latent state in a diverse group of cells that ensures survival of the viral genome throughout the life of the host. Periodic reactivation and viral recrudescence (i.e., appearance with or without clinical symptoms) occur after stress and alterations in immune surveillance. Frank immunosuppression is associated with more severe disease and complications (e.g., encephalitis, pneumonia, hepatitis, and various forms of cancer) (2, 7, 13, 14, 40, 53, 57, 60, 67, 68, 78, 83, 84).

Over 95% of the adult population are infected with HHVs (28, 39, 70, 71, 86). Infections occur following intimate contact with virus-laden secretions and in some case by fomites (e.g., nurseries, day care centers) (22, 72). Saliva harbors most of the HHVs and appears to be an important mode for virus transmission. Although the prevalence of individual HHVs in saliva has been reported, (8, 26), our knowledge of the simultaneous presence of HHVs in saliva of healthy adults who are absent of mucosal pathosis is incomplete. Also, it is unclear whether the pattern of salivary HHV infections changes after stress and whether a prophylactic course of an antiviral drug can alter the pattern and quantity of HHV shedding in saliva. Since dental treatment can be emotionally and physically stressful and influence the outbreak of HSV-1 recurrences (55), we examined these issues in a prospective, randomized, double-blind trial using valacyclovir and placebo in healthy adult patients undergoing dental treatment. In this report, the prevalence, quantity, and duration of HHVs in saliva of individuals over a course of one week and the influence of a two-day course of valacyclovir on the pattern of HHV shedding (i.e., virus in saliva in the absence of symptoms or demonstrable lesions) in patients who experience HSV-1 recurrences and received dental treatment are presented.

MATERIALS AND METHODS

Study population. This study was part of the Valacyclovir Trial for the Prevention of Dentally Induced Cold Sores. The patient population, described elsewhere (55), included men and women 12 years of age or older who had a history of recurrent oral HSV infections, were HSV seropositive, and were scheduled to receive routine dental care at the College of Dentistry, University of Kentucky. All patients were in good general health and did not have histories of liver or kidney dysfunction, symptoms of acute illness (i.e., fever, sore throat, body aches, and diarrhea), or visible oral lesions at the time of enrollment.

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Pregnancy, use of antibiotics, immunosuppressant medications, and antiviral therapy one week before the study enrollment date were additional exclusion criteria. The study was approved by the University Institutional Review Board. All patients provided written informed consent and received incentive (i.e., monetary compensation as well as a clinical exam) as part of the study protocol.

Assay for HSV antibodies. Serum from all patients was tested for anti-HSV antibodies using the IMMULITE herpes I & II immunoglobulin G solid-phase chemiluminescent enzyme immunoassay kit according to the manufacturer's directions (Diagnostic Products Corporation; Los Angeles, CA) by technologists in the University Hospital laboratory.

Dental procedure. Treatment for all patients was planned according to standard procedures employed by the College of Dentistry. The study was initiated for each patient on the first day of the treatment plan sequence. The dental procedures included were periodontal, restorative, endodontic, orthodontic, and oral surgical procedures. Excluded were diagnostic (e.g., clinical examination, radiographic procedures) and prosthodontic procedures.

Study medication. The study medication was valacyclovir (500-mg tablets) or matching placebo. At enrollment, patients were randomly assigned to double-blind treatment and received oral valacyclovir or placebo. The dosage regimen entailed administration of 2 g after sample collection and within 1 h of the dental procedure. Patients were provided a take-home dosage such that they took a second 2-g dose the evening of the dental procedure and then 1 g the next morning and 1 g 8 to 12 h later or matching placebo.

Blinding and assignment. Double-blind study medications were packaged as 12 pills per identical white pill bottle. Patients were assigned sequentially to study medication that was numbered according to a computer-generated randomization code. The treatment blind was maintained and not broken for any patient throughout the trial.

Study design and procedures. The trial was a prospective, randomized, double-blind, placebo-controlled study. At the initial clinic visit (i.e., day dental procedure was completed), informed consent was obtained, a standardized medical history was administered, and an oral examination was performed. Patients then contributed saliva and received randomized study medication and prescribed dental treatment. Patients were asked to report to the clinic on day 3 and day 7 ($\pm$24 h) after the initial dental visit. At each clinical visit, a thorough oral examination was performed, expectorated whole saliva (5 ml) was collected and stored at -80°C until use, and accounts of compliance and any adverse events were obtained. Patients were instructed on how to perform oral lesion assessment and were provided a diary to complete twice daily for eight days. Information recorded in the diary included lesion presence, stage, pain level, adverse events, and compliance. This information is presented elsewhere (55).

PCR primers and probes. The primers and probes used were derived from published sources. Briefly, HSV-1 and HSV-2 primers and probes were designed for glycoprotein G as described by Ryncarz et al. (65). VZV primers and probes were designed to open reading frame 62 (ORF62) as described by Pevenstein et al. (59). Primers and probes for EBV were directed to the BALF5 gene encoding viral DNA polymerase according to Kimura et al. (43). Primers and probes for CMV were designed to glycoprotein B as described in Li et al. (49). Primers and probes for HHV-6 were derived from published data on the U22 open reading frame of HHV-6A strain U1102 as described by Collot et al. (15). Primers and probes for HHV-7 were directed to the major capsid protein as described by Zerr et al. (89). HHV-8 primers and probes were designed to KSHV minor capsid protein as described by White and Campbell (82). Probes for all HHVs were labeled at the 3′ end with the quencher fluorochrome, 6-carboxy-tetramethyl-rhodamine (TAMRA) (PE Applied Biosystems). The 5′ end of each of the probes for all HHVs, except HSV-1, was labeled with the reporter fluorochrome, 6-carboxy-fluorescein (6-FAM). The HSV-1 probe was labeled at the 5′ end with tetrachlorinated analogue of 6-FAM (TET). These primers and probes have been found to reliably detect at least 10 copies of target DNA and are specific when tested with known HHVs (i.e., cross-reactivity is not observed between the viral assays) (15, 43, 49, 59, 65, 82, 89).

Real-time PCR. Real time-PCR was used for the detection and quantification of HHVs in saliva. Saliva samples (1 ml) were centrifuged, and the DNA was isolated from the cell pellet using the QIAamp DNA Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's direction. The DNA yield is typically 5 to 15 μg per ml of saliva using these kits. Each 50-μl PCR mixture contained 10 μl purified DNA template in a final volume consisting of 1× TaqMan Universal PCR master mix (PE Applied Biosystems), 900 nM primers, and 250 nM TaqMan probe. Real-time PCR was performed on an ABI Prism 7700 Sequence Detection system (PE Applied Biosystems). Cycling parameters were 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Each PCR run contained negative controls, including reaction mixtures without DNA template as well as several specimens that were known to contain no HHV DNA, a positive amplicon control, and a 10-fold dilution series (1×10^0 to 1×10^6 genome equivalents per reaction) of either genomic HHV DNA or cloned HHV sequences. The positive control standards were HSV-1 and HSV-2 genomic DNA; the EcoRI-A fragment containing ORF62 of VZV strain Ellen cloned into pGEM kindly provided by S. Straus from NIH, Bethesda, Maryland; plasmid pGEM-BALF5 containing the EBV BALF5 gene kindly provided by H. Kimura, Nagoya University, Nagoya, Japan; plasmid pCR2.1 with a cloned 254-bp fragment of CMV glycoprotein B kindly provided by Y.-W. Tang, Vanderbilt University, Nashville, Tennessee; the pCR1.2 vector containing the U22 gene from HHV-6A kindly provided by S. Collot from Limoges University Teaching Hospital, Limoges, France; the 1e7/μl HHV-7 clone kindly provided by D. M. Zerr, the University of Washington, Seattle, Washington; and plasmid pMCP containing nucleotides 47239 to 47554 of the HHV-8 genome kindly provided by T. B. Campbell, the University of Colorado, Denver, Colorado. Each specimen was analyzed in duplicate. Results were scored positive if both reactions yielded a threshold cycle value above the limit of detection for the standards. Reactions that yielded one positive and one negative result were repeated in duplicate. Samples were scored positive only when both repeat reactions yielded positive results. The copy number results are reported as the means of the two runs.

Statistical analysis. A repeated-measures analysis (Procedure Genmod in PC SAS, version 8E with a logit link, binomial distribution, and repeated statement) was used to determine the log odds that a virus detected varies with time or treatment or the interaction between time and treatment. A multiple logistic model was used to determine the effect of the covariates (i.e., age, race, gender, presence/absence of an invasive dental procedure, and treatment [valacyclovir versus placebo]) on the log odds that the virus was detected at a visit. The second part of the analysis used only detectable responses. These responses were log transformed since the viral copy numbers were highly skewed to the right. A linear mixed model was used to determine the effect of time, treatment, and the interaction between time and treatment on mean response. This was conducted using Procedure Mixed in PC SAS. To determine the effect of the covariates on the log-transformed loads, a multiple regression model was used for the baseline scores with no distinction as to treatment assignment. In both parts of these analyses, statistical significance was determined at the 0.05 level. McNemar's statistic was used to test the hypothesis that some viruses were more common than others. Because of the many comparisons possible, statistical significance was determined at the 0.0018 level to avoid inflation of the type I error rate (i.e., 0.05 divided by 28, the number of statistical tests). The Kappa statistic was used to determine the concordance among the pairs of HHVs being detected within the same sample.

RESULTS

Study population. This study was part of the Valacyclovir Trial for the Prevention of Dentally Induced Cold Sores that intended to determine whether a prophylactic course of valacyclovir therapy could suppress oral HSV recrudescence (55) and/or alter the pattern of HHV shedding in saliva after dental therapy. Of the 150 patients with a history of recurrent oral HSV-1 infections randomized to the study, 125 met the eligibility criteria, took study medication correctly, received one dental procedure, and returned for the two scheduled postoperative visits. None of the participants had detectable lesions of the oral or perioral mucosa at study entry. The 63 evaluable patients in the placebo group and 62 in the valacyclovir group were demographically similar (Table 1).

Presence of HHV DNA in saliva. A total of 375 whole expectorated salivas were obtained from patients collected on the day of the dental procedure prior to drug therapy, and on days 3 and 7 post-dental treatment (PDT). Saliva was centrifuged, and the DNA extracted from the cell pellet was analyzed. We chose to analyze the cell pellet inasmuch as some herpesviruses are highly cell associated and were detected more readily in the cell pellet than the supernatant of representative samples (data not shown). Sufficient DNA was available from 364 samples for analysis using real-time PCR. We detected 10 copies of HSV-1,

TABLE 1. Demographics of study population

Group	Value for group treated with:		Overall
	Placebo (n = 63)	Valacyclovir (n = 62)	
No. (%) Female	43 (68.3)	42 (67.7)	85 (68)
No. (%) Male	20 (31.7)	20 (32.3)	40 (32)
No. (%) of race:			
White	56 (88.9)	59 (95.2)	115 (92)
Black	6 (9.5)	3 (4.8)	9 (7.2)
Other	1 (1.6)	0 (0)	1 (0.8)
Mean age in yr (range)	38.7 (12–79)	38.8 (13–68)	38.5 (12–79)

HSV-2, VZV, CMV, and HHV-6 target sequences per reaction and 1 copy of EBV, HHV-7, and HHV-8 DNA sequences in the assays performed. Negative controls did not generate a product in any assay.

HHV DNAs were detected in the saliva of every patient and 100% of the 364 samples. At the initial visit (i.e., before treatment; Fig. 1), HHV-6 and HHV-7 were detected significantly more often than the other viruses ($P < 0.002$). EBV was detected at baseline in 43.1% of the population, HSV-1 in 8.9%, HHV-8 in 0.8%, and CMV in 0.8%. HSV-2 and VZV were not detected. Although analyses for HSV-2 and VZV were negative in all specimens, DNA specific for these viruses was detected in the positive controls by real-time PCR.

EBV and HHV-6 were slightly more prevalent in men (50% and 97.5%, respectively) than women (42.2% and 91.6%, respectively) (data not shown). Results of multiple logistic regression showed that with only one exception none of the covariates age, gender, and race was a significant predictor of the log odds that an HHV was detectable at baseline. The exception was age for HSV-1, with the mean age of expressors (28.1 years) significantly less ($P = 0.02$) than that of nonexpressors (39.7 years) (data not shown).

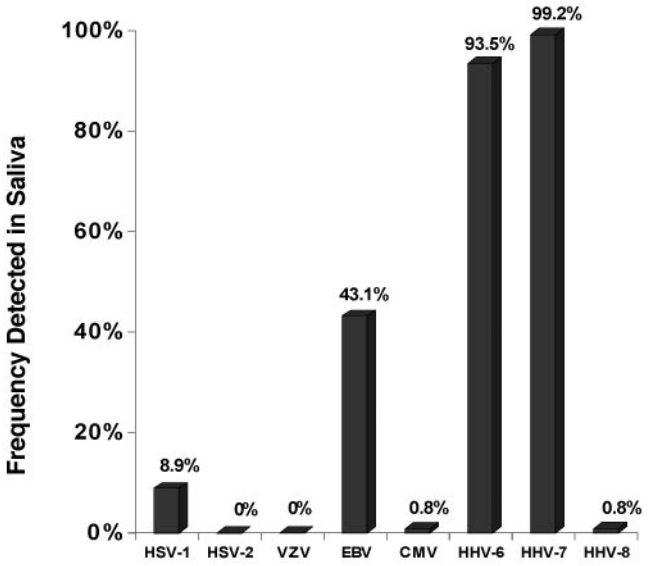

FIG. 1. Prevalence of HHVs in saliva of healthy immunocompetent individuals with a history of recurrent oral HSV infections at the initial visit. The DNA from the cellular fraction of saliva of 123 dental patients was isolated and amplified in duplicate using real-time PCR as described in Materials and Methods.

Over the course of the week, the DNAs of HHV-6 and HHV-7 were detected significantly more often (97% to 99% of patients, respectively) than EBV (64.8%), HSV-1 (13.0%), HHV-8 (3.2%), CMV (2.4%), HSV-2 (0%), and VZV (0%) ($P < 0.002$). Coinfections occurred in 99.2% of patients. HHV-6 and HHV-7 were simultaneously present in 93.6% of specimens. EBV coinfected saliva with HSV-1 in 3.8%, HHV-6 in 38.5%, and HHV-7 in 40.1% of specimens. At least three HHVs were detected simultaneously in 36.5% of specimens, and 4.8% of specimens were coinfected with four viruses. We did not detect DNA from more than four HHVs in any specimen.

Pattern of viral shedding following dental manipulation. Table 2 demonstrates the prevalence of HHVs in the placebo group did not change substantially following dental treatment. In these patients, HHV-7 was present in 99.2% of salivas collected from all three visits, HHV-6 was present in 95.1%, 94.8%, and 95%, EBV was present in 41%, 46.6%, and 48.3%, and HSV-1 was present in 9.8%, 10.3%, and 8.3%, respectively. At the postoperative visits, CMV and HHV-8 were detected in 0% to 3.3%, whereas HSV-2 and VZV were not detected in any salivas analyzed. Specific dental procedures were not associated with the detection of any HHV at the postoperative visits.

The frequency of detection of HHV DNAs at multiple appointments for the population is shown in Fig. 2. Only HHV-6 and HHV-7 were detected in the majority of patients at more than one appointment. At any two visits, HHV-7 was detected in 95.2% of patients, HHV-6 in 85.6%, EBV in 38.4%, and HSV-1 in 1.6%. The frequency of detection at three consecutive visits remained high for HHV-6 and HHV-7 but was less than 20% for the remaining HHVs. The covariates age, gender, race, and invasiveness of procedure were not significant predictors of shedding of any HHV at postoperative or multiple visits.

Quantitative analysis of HHV DNA in saliva of expressors in the placebo group. Concentrations of HHV DNA varied greatly in the expressors, from 2 to 53,784,455 copies per ml of saliva for HHV-7 (median, 1,779), 10 to 9,474,044 for HHV-6 (median, 1,917), 69 to 2,069,855 for HSV-1 (median, 45,000), 1 to 49,484 for EBV (median, 117), 12 to 558 for CMV (median, 354), and 10 to 410 for HHV-8 (median, 34). In the expressors of the placebo group, the highest mean copies were for HSV-1 and HHV-6, with an average of 414,005 and 66,277 copies detected per ml of saliva, respectively. Mean genome copy numbers of HHV-7 and EBV were 14,369 and 1,418 per ml of saliva, respectively. HHV-8 was detected in low quantity in the expressors (mean, 122 copies/ml), whereas HSV-2, VZV, and CMV were not detected in any patient of the placebo group.

Table 2 shows that dental manipulation did not significantly alter the geometric mean copy number of any HHV measured from the initial visit to days 3 and 7 PDT in the placebo group. When the logs of the viral copies of paired HHVs were examined, a significant correlation between HHV-6 and HHV-7 was observed (Pearson's coefficient; $r = 0.31$; $P < 0.0007$).

TABLE 2. Geometric mean genome copy numbers in saliva of the expressors

Virus and treatment	Result of:					
	Visit 1		Visit 2		Visit 3	
	% (*n*/total)	Geometric mean copy no.	% (*n*/total)	Geometric mean copy no.	% (*n*/total)	Geometric mean copy no.
HSV-1						
Placebo	9.8 (6/61)	17,034	10.3 (6/58)	22,035	8.3 (5/60)	596
Valacyclovir	8.1 (5/62)	3,419	1.6 (1/62)	8,978	6.6 (4/61)	2,254
EBV						
Placebo	41.0 (25/61)	97	46.6 (27/58)	64	48.3 (29/60)	89
Valacyclovir	45.2 (28/62)	84	29.0 (18/62)	55	34.4 (21/61)	123
CMV						
Placebo	0 (0/61)	0	0 (0/58)	0	0 (0/60)	0
Valacyclovir	1.6 (1/62)	558	0 (0/62)	0	3.3 (2/61)	83
HHV-6						
Placebo	95.1 (58/61)	1,851	94.8 (55/58)	2,205	95 (57/60)	2,284
Valacyclovir	91.9 (57/62)	1,537	91.9 (57/62)	1,556	93.4 (57/61)	2,174
HHV-7						
Placebo	100 (61/61)	3,618	100 (58/58)	3,238	98.3 (59/60)	2,686
Valacyclovir	98.4 (61/62)	3,285	98.4 (61/62)	3,430	96.7 (59/61)	4,624
HHV-8						
Placebo	0 (0/61)	0	0 (0/58)	0	3.3 (2/60)	35
Valacyclovir	1.6 (1/62)	410	1.6 (1/62)	10	0 (0/61)	0

Effect of valacyclovir on HHV shedding in saliva. The proportion of patients shedding virus in saliva before and after drug therapy is shown in Fig. 3. On the day of study initiation, the proportions of patients shedding HHVs were similar in the two groups. However, fewer patients shed HSV-1 and EBV after valacyclovir treatment. The percentage of patients who shed HSV-1 in the valacyclovir group decreased from 8.1% (initial visit) to 1.6% on day 3 PDT and subsequently increased to 6.6% on day 7 PDT. The prevalence of EBV decreased from 45.2% (initial visit) to 29% (day 3 PDT) following valacyclovir treatment ($P < 0.05$). The effect was greatest in the age group 50 years and older, where the prevalence of EBV dropped from 43% to 12% following antiviral therapy ($P = 0.22$ by McNemar's statistic) (data not shown). On day 3 PDT, significantly fewer patients who took valacyclovir (29%) shed EBV than those who took placebo (46.6%; $P = 0.05$). Valacyclovir treatment also resulted in significantly fewer adults (18%) who shed EBV at consecutive postoperative appointments compared with placebo (40.3%; $P = 0.008$; chi square) (data not shown). For each treatment group and all HHVs, the geometric mean genome copy numbers did not change significantly between the initial and postoperative visits.

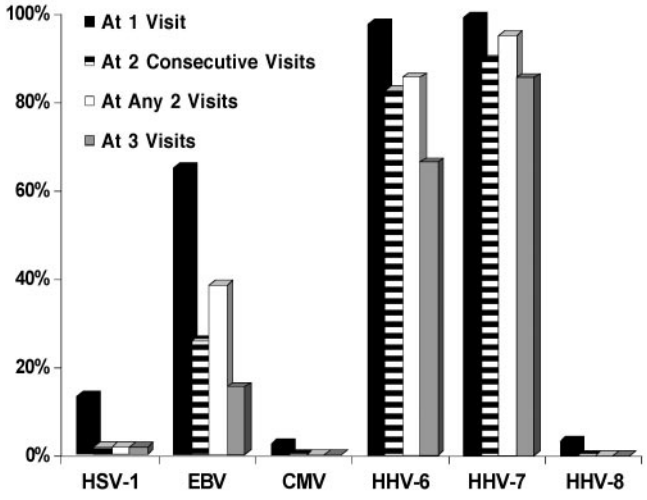

FIG. 2. HHV shedding in saliva during consecutive visits and the week after dental treatment.

DISCUSSION

This is the first study to evaluate the prevalence, pattern, and effect of dental and antiviral treatment on the excretion and quantitative levels of HHVs in saliva of immunocompetent adults who had a history of recurrent oral HSV-1 infections. The findings indicate that HHVs frequently coexist and are repeatedly released into the oral cavity of the majority of healthy adults in the absence of clinical signs and symptoms, HHV-6 and HHV-7 are highly prevalent in saliva, and the rate of asymptomatic shedding of HHVs does not increase significantly after periods of oral trauma (i.e., dental manipulation). Further, a short course of an antiviral agent reduced the prevalence of a single HHV type (i.e., EBV) in saliva for several days after dosing.

To date, our understanding of the reported patterns of HHV shedding in saliva of healthy individuals is limited by studies that have used methods with various sensitivities and examined a limited number of HHVs or a small numbers of patients. When large numbers of patients have been studied, the age

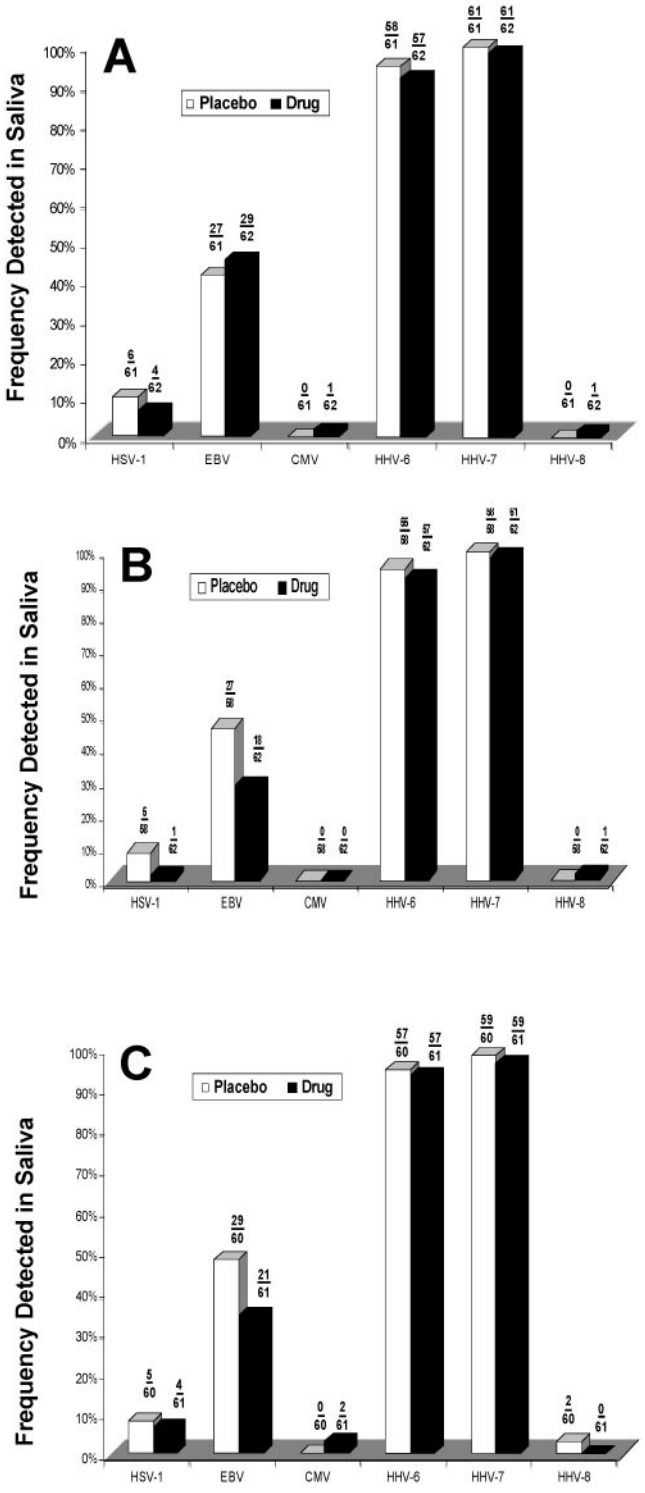

FIG. 3. Effect of valacyclovir therapy on HHV shedding in saliva. (A) Visit 1. (B) Visit 2. (C) Visit 3.

and health status have not always been documented, sampling has been relatively infrequent or often performed at a single visit, and oral health status has not been rigorously evaluated or affected by dental treatment. Current evidence indicates that the mean detection rates of HHVs in the oral cavity of healthy adults at a single visit are as follows: HHV-7, 79%; HHV-6, 62%; EBV, 31%; VZV, 10.8%; HSV-1, 6.0%; HSV-2, 0.6%; CMV, 0.6%; and HHV-8, 0% (1, 3, 6, 8-10, 15, 17, 19, 20, 23, 24, 27, 29-38, 41, 42, 44, 47, 48, 50-52, 58, 61, 66, 69, 73, 75, 76, 85, 88, 89). For the majority, our prevalence figures are similar to findings from investigations that have examined large patient samples using PCR. At the initial appointment, we detected HHV-7 in 99.2% of samples, HHV-6 in 93.5%, EBV in 43.1%, HSV-1 in 8.9%, CMV in 0.8%, HHV-8 in 0.8%, and HSV-2 and VZV in 0%. In the placebo group, the rate of asymptomatic HHV shedding did not change significantly during the week following dental treatment.

Of the demographic factors analyzed, only age was correlated with salivary shedding of a HHV at baseline. Specifically, the mean age of expressors of HSV-1 (28.1 years) was significantly less than that of the nonexpressors (39.7 years). This is consistent with reports that HSV-1 is shed in saliva more often by infants and children than adolescents and adults (9, 76) and extends the findings to suggest that young adults are more likely to excrete HSV-1 than older adults.

The duration of HHV shedding is profiled in Fig. 2. The data suggest that HHV-6 and HHV-7 were consistently present in the saliva of most adults, EBV was present at more than one visit in 38% of patients, and HSV-1 was detected infrequently (1.6%) at consecutive visits. CMV and HHV-8 were not detected at consecutive appointments in any patient, and HSV-2 and VZV were not detected in any salivas. Overall, these data are consistent with the findings of others who have examined the duration of HHV shedding in saliva (21, 32, 38, 50, 88) and indicate that the prevalence of HHVs in the saliva of asymptomatic adults does not increase upon the stress of dental treatment.

We are unaware of any other studies that have quantified, during one week, the genome copy numbers of all HHV types in saliva of healthy, immunocompetent adults who had a history of recurrent oral HSV infections. Therefore, in these regards, our data are novel. Our median copy number yields for positive samples are similar to values reported by several investigators (8, 15, 17, 27, 42, 66) but lower than that reported by Walling et al. (80) for EBV (1,110,000 in 0.5 μg of DNA). Differences could be attributed to selection bias from their use of smaller numbers of patients, regional population differences, or the facts that we examined HSV-seropositive individuals, samples were freeze-thawed, and DNA was analyzed from the cellular fraction of saliva. Although several investigators have used whole saliva (cells and fluid) for analyses (1, 17, 42), we found a slight advantage in the ability to detect HHV DNA by the described method using the cellular over supernatant fractions (data not shown).

Our real-time PCR procedures yielded sensitivity levels of 1 to 10 copies that resulted in the detection of six of the eight HHVs in the saliva of healthy adults. Not surprisingly, the prevalence and yields were low for CMV and HHV-8 and less than the values reported from individuals with immunological disorders, men who have sex with men, sex workers, and persons who had Kaposi's sarcoma (11, 19, 45, 46). Of note, genome copy numbers of HSV-1, EBV, HHV-6, and HHV-7 were high (>1,000 copies/ml) on at least one visit for many patients. These high DNA concentrations in saliva are sugges-

tive of ongoing virus replication in asymptomatic individuals and are consistent with previous reports concerning asymptomatic excretion of select HHVs at oral (10, 45, 74, 75, 79) and extraoral (5, 64) sites. Interestingly, the geometric mean genome copy numbers of the eight HHVs did not change significantly in the placebo group whether or not dental manipulations were rendered. This appears to suggest that the stress experienced in this study did not enhance the replication of HHVs within the oral milieu of asymptomatic, lesion-free individuals.

An interesting observation of this study was the duration of effect of valacyclovir on asymptomatic HHV shedding. A two-day, prophylactic course of valacyclovir resulted in a diminished proportion of patients excreting EBV in saliva for at least two days after drug administration. Antiviral therapy also resulted in lower (arithmetic) genome copy numbers of HSV-1 and EBV (data not shown) and significantly fewer adults who shed EBV at consecutive appointments compared with placebo ($P = 0.03$). These findings are consistent with the observed effects of acyclovir and valacyclovir on HSV and EBV replication (4, 16, 18, 54, 77, 87) and clearly demonstrate that the antiviral benefit extended a few days after administration.

In summary, this study shows that in the saliva of healthy individuals EBV, HHV-6, and HHV-7 are frequent and persistent, HSV-1 is intermittently and temporarily present (i.e., less than 3 days), CMV and HHV-8 are episodically present, and HSV-2 and VZV are rarely present. The high genome copy numbers of HSV-1, EBV, HHV-6, and HHV-7 suggest that frequent reactivation or chronic infection (25, 56) may be contributory. Clearly, their presence could facilitate transmission. The effectiveness of valacyclovir in reducing the prevalence of EBV in saliva suggests that antiviral agents could provide one approach for limiting the presence of these viruses in the oral cavity.

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