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Detection of Viral DNA To Evaluate Outcome of Antiviral Treatment of Patients with Recurrent Genital Herpes

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Culture of infectious virus, PCR amplification of viral DNA, and the appearance of genital skin lesions were used as markers to study the course of a recurrence of genital herpes in 40 patients treated with famciclovir or placebo. The highest frequency of patients with skin lesions occurred within the first 36 h following the onset of a recurrence, which also corresponded to the peak in the production of virus. While the timing of the peak in skin lesions was independent of the type of treatment, the frequency of lesions and the release of virus at the lesion site were both reduced by famciclovir treatment. Furthermore, patients receiving this antiviral agent showed a more rapid recovery time and a shorter period during which viral DNA could be detected at the lesion. PCR and then Southern blot hybridization greatly enhanced our ability to detect herpes simplex virus at the lesion site. This procedure proved to be of greater diagnostic value in assessing genital herpes than the standard culture method currently used. In addition, PCR was more sensitive in evaluating treatment effectiveness.

Genital herpes, resulting from herpes simplex virus (HSV) type 1 (HSV-1) or HSV-2 infection of the urogenital tract, is a worldwide public health problem. Several antiviral drugs have been used in the treatment of primary infections and recurrences but with variable results. Although several candidate vaccines are in clinical trials, no vaccine is available for general use.

Herpetic lesions will, in the majority of cases, cause severe discomfort and will have detrimental effects on the healthy sexuality of infected adults. In addition, a high percentage of neonates who contract HSV-1 or HSV-2 at birth die or suffer severe neurological damage, despite antiviral therapy (1, 24, 25). The rate of neonatal HSV infection could be significantly reduced by the development of sensitive, specific, and rapid screening procedures which could identify babies at risk and which could allow for the planning of an alternative route of delivery or for the early initiation of antiviral treatment following delivery. Such procedures are also required in order to follow the effects of new drugs on the frequency of herpetic recurrences and on viral shedding during acute or recurrent infections.

While tissue culture has remained the standard procedure for the detection of HSV, it requires at least 2 to 3 days before results can be obtained and a minimum titer of infectious virus (1, 3, 9, 15). Detection of viral antigens by immunological methods has also not yet achieved the desired sensitivity (4, 21). PCR, on the other hand (17), has high degrees of sensitivity and specificity for the detection of selected viral DNA sequences. Although it does not differentiate between infectious and noninfectious virus, it has been used successfully to identify human immunodeficiency virus, human papillomaviruses, and cytomegalovirus (14, 19, 20) and also to study populations at high risk of shedding HSV-1 and HSV-2 (5–7, 10, 11, 16). To date, however, PCR has not been used to follow the evolution of HSV infection in patients with recurrences of genital herpes or in patients with genital herpes on antiviral treatment. Here we report the use of PCR and virus culture to follow the natural history of recurrent genital herpes, virus and viral DNA shedding, and the association of this shedding with different stages of skin lesions. The effects of famciclovir, an inhibitor of the HSV DNA polymerase (22, 23), on these parameters were also analyzed.

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MATERIALS AND METHODS

Patient population. The study was approved by the Human Experimentation Committee at the Ottawa General Hospital, University of Ottawa. The patients were selected as part of a multicenter study to evaluate the efficacy and safety of three different doses of famciclovir for the treatment of recurrent genital herpes. All the data collected from patients enrolled in the Ottawa center are presented in this report. The recruited patients were otherwise healthy and nonpregnant individuals who were over the age of legal consent and who had a previous diagnosis of genital herpes proven by culture. All patients in our study group had recurrent episodes of genital herpes at least every 4 months with a clearly identifiable prodrome. Patients were instructed to self-culture the skin site where the lesions or prodrome occurred. The initial swab was taken within 6 h of the appearance of lesions or prodrome, and treatment was started immediately thereafter. The patients reported to the clinic within 12 h of the recurrence for initial clinical assessment and were assessed twice daily for 5 days and once daily thereafter until complete reepithelialization of the genital lesion occurred or only until day 5 if the lesion was healed. At each visit, the symptoms and the conditions of the lesions were documented and samples were collected for virus culture and DNA amplification. A total of 40 patients were recruited into the study; 10 patients received placebo and the other 30 patients received famciclovir. The treatment regimen consisted of 125 mg (10 patients), 250 mg (13 patients), and 500 mg (7 patients) of oral famciclovir administered twice daily for 5 days following the appearance of lesions or prodrome.

Clinical observations. The prodromal areas or lesion sites were examined, and skin lesions were recorded as papules, vesicles, ulcers, and/or crusts.

Viral studies. Swabs were taken from the genital prodromal areas or lesion sites and were soaked in preparative medium (Iscove's modified Dulbecco's medium [Gibco/BRL] supplemented with 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 0.2% bovine serum albumin, 25 μg of amphotericin B per ml, 1,000 IU of penicillin per ml, and 500 μg of gentamicin per ml) to elute virus. An aliquot (0.1 ml) of this suspension was used to inoculate subconfluent cultures (80% confluence) of a human lung fibroblast cell line growing in Dulbecco's modified Eagle medium (Gibco/BRL) supplemented

with 2% fetal calf serum. The cultures were kept at 37°C for 1 to 7 days until about 50 to 75% of the monolayer showed cytopathic effects. The cultures were then harvested for analysis by immunofluorescence. The Micro Trak HSV-1 and HSV-2 Culture Identification/Typing Test (Syva, San Jose, Calif.), which uses two fluorescein-labelled monoclonal antibodies directed against the HSV-2 ribonucleotide reductase and glycoprotein B doublet, were used to type the HSV isolates in the cultures. Cultures without a cytopathic effect were discarded after 7 to 8 days and were reported as negative.

In order to determine the one-half tissue culture infective dose ($TCID_{50}$), subconfluent human lung fibroblast cell cultures were inoculated with 0.1 ml of serial 1/10 dilutions of the HSV-1 McIntyre strain (ATCC VR539) and HSV-2 strain V58399 (clinical isolate). After 7 days, the cultures were harvested and their $TCID_{50}$s were determined (12).

PCR conditions and primers. A duplicate swab was taken at the same time and from the same site as that used for the viral culture. This second swab was placed in 300 μl of lysis buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2.5 mM $MgCl_2$, 0.5% Tween 20, 0.5% Nonidet P-40). The samples were digested at 37°C overnight with 100 μg of proteinase K and were then boiled for 10 min to inactivate the enzyme. The viral DNA extracts were stored at −20°C until they were tested by PCR. The oligonucleotide primers used were designed to amplify a portion of the HSV DNA polymerase gene (13). The upstream primer DNAP5 recognizes both HSV-1 and HSV-2 sequences, while the downstream primers DNAP3-1 and DNAP3-2 recognize nucleotide sequences of HSV-1 and HSV-2, respectively. The oligonucleotide internal probe HSV-P3 recognizes both HSV-1 and HSV-2 sequences. By using these primers, we were able to distinguish between the presence of HSV-1 and HSV-2 on the basis of the PCR product size, 469 and 391 bp, respectively (13). PCR was carried out for 35 cycles in a programmable DNA thermal cycler (9600; Perkin-Elmer), as follows: DNA denaturation at 94°C for 40 s, primer annealing at 65°C for 1 min and 30 s, and primer extension at 71°C for 2 min. For the last cycle, the DNA molecules were allowed to extend for 7 min at 72°C. The amplified DNAs were then detected by electrophoresis on a 1.5% agarose gel and were stained with ethidium bromide (18).

In order to determine the sensitivity of the PCR method, HSV-1 and HSV-2 stocks were grown in human lung fibroblast cells and their $TCID_{50}$s were determined by the Reed and Muench method described previously (17). Serial 10-fold dilutions of culture supernatants containing titers of 10^7 $TCID_{50}$s/ml for HSV-1 and 10^4 $TCID_{50}$s/ml for HSV-2 were extracted to isolate viral DNA, and each sample was subjected to PCR amplification and then analyzed by ethidium bromide staining and Southern blot hybridization.

Clinical samples from patients with genital lesions but negative by PCR were spiked with HSV-2 DNA to rule out the presence of PCR inhibitors.

Southern blotting. PCR products were transferred (18) to nylon filters (Amersham) and were hybridized with the oligonucleotide probe HSV-P3 (13). This probe was labelled with the E-LINK Oligonucleotide Labelling Kit (Cambridge Research Biochemicals). Prehybridization of the filters was done for 4 h at 42°C with 10 ml of prehybridization buffer (5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.3% blocking buffer [Boehringer Mannheim, Laval, Quebec, Canada], 0.1% sarcosyl, and 0.01% sodium dodecyl sulfate [SDS]). Hybridization was performed at 42°C overnight with 10 ml of prehybridization buffer plus and 20 μl of the E-LINK probe. After successive washes with 2× SSC plus 0.1% SDS, 0.5× SSC plus 0.1% SDS, and buffers A (100 mM Tris [pH 7.4], 150 mM NaCl), B (2% blocking buffer in buffer A), and C (100 mM Tris [pH 9.5], 100 mM NaCl, 50 mM $MgCl_2$), the damp filters were sprayed two or three times with LumiPhos 530 (Boehringer Mannheim) and were exposed to X-ray film (X-Omat; Kodak) at 37°C for between 30 min and 2 h.

Statistical methods. Analysis of variance was used to compare the different treatment groups. In addition, weighted kappa statistics (using quadratic weights) were used to compare the agreement between the presence of genital lesions and the results of DNA PCR and virus culture, since a Pearson *r* value gives an overly optimistic value in situations in which agreement is being evaluated. Since antiviral treatment inhibited both virus and viral DNA shedding, this analysis was undertaken only for subjects in the placebo group. Means were also compared by two-tailed Student's *t* test. Proportions were compared by Fisher's exact test.

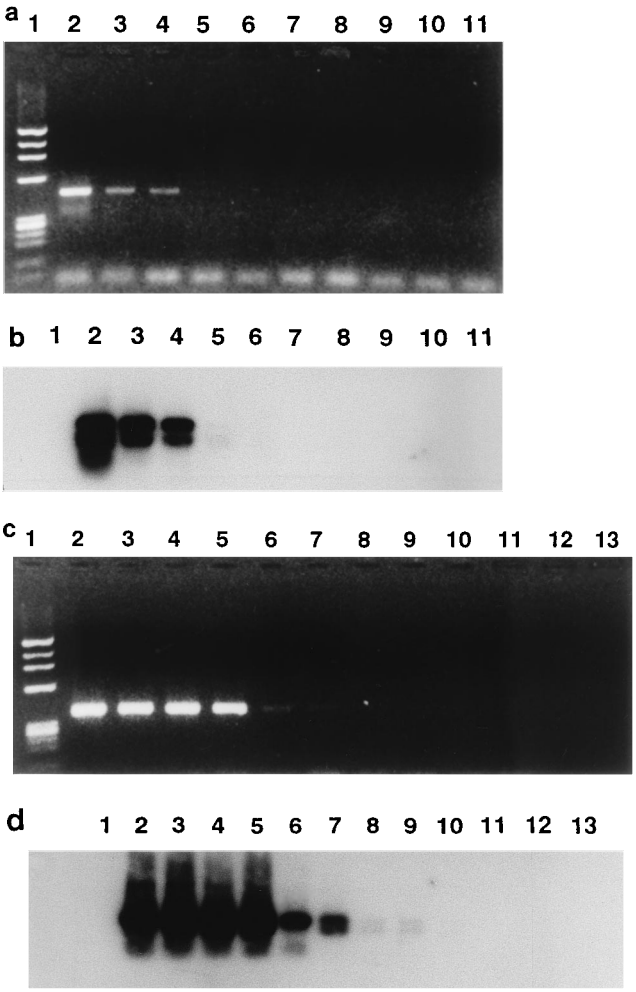

FIG. 1. Sensitivity of HSV PCR. An aliquot (100 μl) of the supernatants from cultures of HSV-1 (a and b) and HSV-2 (c and d), with titers of 10^7 and 10^4 $TCID_{50}$s/ml, respectively, were extracted, diluted 10-fold, and amplified as described in Materials and Methods. Samples from the PCR were then electrophoresed in agarose and stained with ethidium bromide (a and c) or transferred to nylon filters and hybridized with the labelled probe (b and d; see Materials and Methods). The last positive dilutions after hybridization with the labelled probe were 10^{-10} for HSV-1 and HSV-2, which represents limits of detection of 10^{-3} and 10^{-6} for HSV-1 and HSV-2, respectively. Lanes 1, phage 174 replicative-form DNA digested with *Hae*III as a size marker; lanes 2 to 11 (a and b), 1/10 serial dilutions of HSV-1 (10^{-6} to 10^{-15}); lanes 2 to 12 (c and d), 1/10 serial dilutions of HSV-2 (10^{-3} to 10^{-13}); lanes 13 (c and d), negative control containing all components of the PCR except target DNA.

RESULTS

Sensitivity of the PCR procedure. The comparison of PCR with virus culture demonstrated that PCR could detect viral DNA in dilutions beyond the point at which infectious virus was detectable. As shown in Fig. 1, herpesvirus DNA could be detected with the HSV-P3 probe in culture supernatants diluted as low as 10^{-10} for HSV-1 and HSV-2. This represents a limit of detection of much less than 1 $TCID_{50}$/ml for both viruses (10^{-3} $TCID_{50}$s/ml for HSV-1 and 10^{-6} $TCID_{50}$s/ml for HSV-2). Hybridization of the PCR products with the HSV-P3 probe increased the sensitivity of the assay with respect to ethidium bromide staining by a factor of 10^2 (Fig. 1). Hybridization of clinical samples amplified by PCR with the labelled oligonucleotide probe increased from 28 to 34 the number of patients in whom viral DNA could be detected, thus increasing the sensitivity by 15% (see Table 1). The use of this procedure allowed the detection of viral DNA in 100% of the patients treated with placebo and even allowed the detection of viral DNA in one patient who had prodromic symptoms but who did not develop skin lesions (see below).

Clinical symptoms and their association with virus detection and viral DNA amplification during the course of recurrent genital herpetic lesions. Virus culture and DNA amplification were performed in 363 samples collected from 40 patients (Table 1). The patients were divided into four groups according to their treatment: those receiving placebo or 125, 250, or 500 mg of oral famciclovir administered twice daily. Swabs were taken at 12-h intervals for 5 days after the begin-

TABLE 1. Skin lesions, virus isolation, and DNA amplification in patients with a recurrence of HSV-2 treated with famciclovir or placebo

Treatment group	Total[a]		Skin lesions[b]		Virus isolation[c]		PCR (EtBr)[d]		PCR + hybridization[e]	
	No. of patients	No. of samples	No. of patients	No. of samples	No. of patients	No. of samples	No. of patients	No. of samples	No. of patients	No. of samples
Placebo	10	100	9 (90)[f]	71 (71)	8 (80)	32 (32)	9 (90)	59 (59)	10 (100)	67 (67)
Famciclovir										
125 mg	10	89	8 (80)	45 (50.6)	5 (50)	9 (10.1)	7 (70)	17 (19.1)	8 (80)	25 (28)
250 mg	13	102	10 (76.9)	66 (64.7)	5 (38.4)	10 (9.8)	7 (53.8)	19 (18.6)	9 (69.2)	23 (22.5)
500 mg	7	72	7 (100)	51 (70.8)	5 (71.4)	16 (22.2)	5 (71.4)	23 (31.9)	7 (100)	31 (43)
Total treated	30	263	25 (83.3)	162 (61.6)	15 (50)	35 (13.3)	19 (63.3)	59 (22.4)	24 (80)	79 (30)

[a] Number of patients participating in the study and total number of samples collected for each treatment.
[b] Patients with genital lesions and total number of samples taken from genital lesions.
[c] Patients infected with virus that grew in culture during the course of the study and number of samples in which HSV-2 was isolated.
[d] Patients from whom HSV-2 DNA was detected by PCR amplification and samples positive by ethidium bromide (EtBr) staining of the gels.
[e] Patients with positive results after PCR and Southern blot hybridization and number of samples positive by this procedure.
[f] The results in parentheses represent percent positive.

ning of the prodrome or recurrence and then once a day until reepithelialization occurred. The mean number of samples per patient was 9 (range, 5 to 16). Figure 2 summarizes the time course of the development of skin lesions as well as the time course of virus and viral DNA shedding during a recurrence of genital herpes in the population of patients treated with placebo. Both the detection of HSV DNA and the ability to culture virus were related to the stage of the lesion, with a higher number of positive results being found for samples taken from vesicles or ulcers. The peak in virus shedding, as indicated by a positive culture test results, was observed within the first 24 h of the development of skin lesions coincident with the peak in the amplification of viral DNA by PCR. After day 1, there was a slow decay in the level of infectious virus cultured, whereas the presence of viral DNA detected by PCR remained high (70%) until day 4 (samples 6 to 8). As the level of detectable viral DNA declined, so did the frequency of skin lesions.

Agreement between last day with lesions and last day with positive test result. To compare the diagnostic value of viral DNA detection and virus culture in the assessment of genital lesions, we measured the correlation between the last day that a positive test result was observed and the last day that lesions were present. The kappa value was 0.51 for agreement between the virus culture method and the presence of lesions and 0.72 for agreement between DNA PCR results and the presence of lesions.

Effects of famciclovir treatment on herpetic lesions. Patients on famciclovir had lower rates of skin lesions and virus shedding than patients on placebo. In patients receiving placebo, 67% of the samples collected during the course of the study were positive by PCR, while in famciclovir-treated patients the percentage of positive samples by PCR was 30% (Table 1). Patients on placebo had skin lesions at 71% of the time points studied, while patients on famciclovir had skin lesions at 61.6% of the time points studied (Table 1). The data obtained from patients receiving either of the three doses of famciclovir (Table 1) were pooled and analyzed (Fig. 3). In comparison with the placebo group, patients in the famciclovir group had a delayed onset of skin lesions when they presented with a recurrence. In the first 12 h of the recurrence, 8 of 10 patients (80%) in the placebo group had vesicular lesions, whereas 15 of 40 patients (37.5%) in the famciclovir group had vesicular lesions ($P < 0.02$) (Fig. 2 and 3). Similarly, in the first 24 h, the rate of shallow genital ulcers was lower in the famciclovir group than in the placebo group (1 of 40 versus 5 of 10, respectively; $P < 0.001$). Patients on the antiviral treatment did, however, show an increase in the rate of genital lesion formation between 24 and 36 h following the initiation of symptoms. This peak immediately subsided, with the frequency of symptoms dropping at a faster rate than that for the control group (Fig. 3). For both the placebo control and famciclovir groups, there was an apparent plateau in the decline of skin lesions at about day 4 (samples 6 to 8). Nevertheless, famciclovir-treated patients showed a trend for faster recovery of skin lesions than patients treated with placebo (Fig. 2 and 3).

Famciclovir consistently inhibited infectious virus and viral DNA from clinical samples. A consequence of this inhibition was the widening of the gap between the frequency of virus and viral DNA detection and the frequency of skin lesions as seen at the later time points (Fig. 3). The detection of infectious virus in patients on famciclovir reached its highest frequency on day 1, as was the case for placebo-treated patients. However, while for patients on placebo the highest virus isolation rate was in the second sample taken on day 1 (sample 1, 40%; sample 2, 70%), for famciclovir-treated patients a more constant rate was maintained over the same time period (sample 1, 32%; sample 2, 30%). In contrast to infectious virus, the level of viral DNA detected by PCR peaked in both groups at about 24 h following the onset of symptoms. Two marked differences, however, are apparent when comparing the control and famciclovir groups. First, the frequency of virus shedding is consistently and significantly lower in the treatment group. Even when using the more sensitive PCR assay on samples taken at the 24-h time point, only 63.3% of the patients in the treatment group showed evidence of viral DNA at the lesion site, whereas 90% of the patients receiving placebo showed evidence of viral DNA at the lesion site. Second, patients on placebo had persistent infectious viral detection rates of 30 to 40% from day 1 to day 3; this was followed by a slow decline through day 5, falling to about 10%. Patients on famciclovir, on the other hand, had a 10-fold decrease in the presence of infectious virus over the first 3 days, dropping from 34% to 3% of shedding infectious virus. No further virus was detected by culture after sample 7 (3.5 days) in the treatment group (Fig. 3).

For those patients receiving famciclovir, reepithelialization of visible skin lesions occurred within 6 to 7 days following the initiation of treatment. Viral DNA was not detected by PCR in this group beyond day 5. In the control group, herpetic lesions

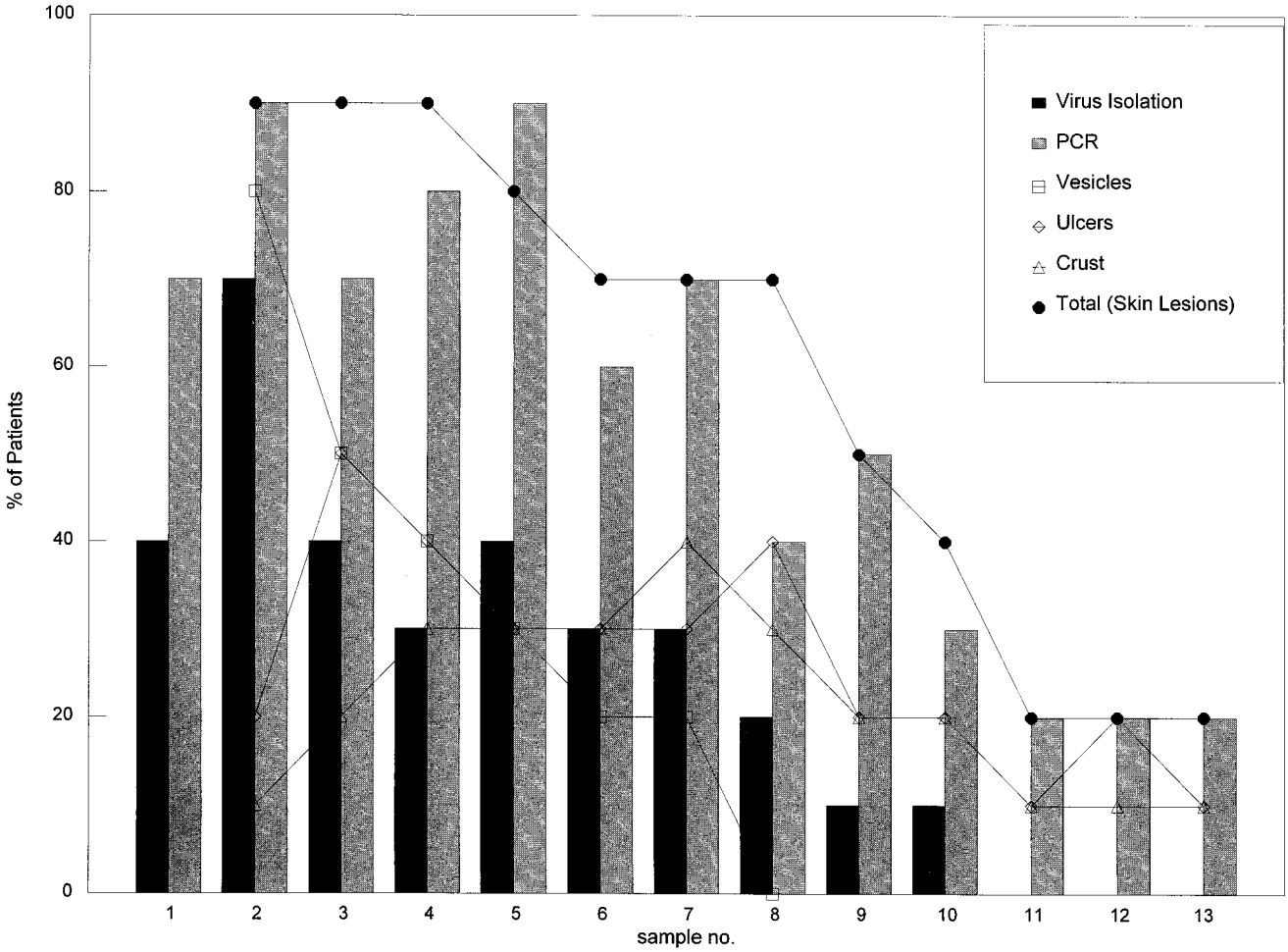

FIG. 2. Skin lesions, virus isolation, and DNA shedding in 10 patients with a recurrence of genital HSV-2 treated with placebo. Prodromal areas or lesion sites were examined, and samples were collected every 12 h for 5 days (samples 1 to 10) and every 24 h thereafter (samples 11 to 13). Sample 1 was collected by the patient before treatment. Samples 2 to 13 were collected by a nurse after treatment was initiated. Lesions were recorded as vesicles, ulcers, or crusts. The total number of patients with skin lesions at each time point is shown. The bars represent virus isolation in tissue cultures or DNA detection after PCR and then Southern blot hybridization with the labelled probe (see Materials and Methods).

persisted in 2 of the 10 patients through day 8. These patients also continued to have detectable viral DNA at the lesion sites.

There was no apparent variability in the mean duration of lesions with respect to drug dosage. The mean duration of virus shedding as measured by culture was 2.05 days for the placebo group and 0.88 days for the famciclovir group ($P < 0.02$). The time frame, then, during which infectious virus could be cultured from the lesion site was shorter than the mean duration of the lesion, indicating that virus shedding declines prior to reepithelialization. However, when lesion sites were examined for the presence of HSV by PCR, viral DNA could be consistently detected at the lesion site within the control group. The mean duration of the presence of viral DNA was 4.3 days for the placebo group and 2 days for the famciclovir group ($P < 0.001$), which are very close to the mean durations of the lesions, which were 3.8 and 2.9 days for the placebo and treatment groups, respectively ($P = 0.17$). Use of the more sensitive PCR method, then, indicates the presence of virus or viral products at the lesion site for a longer time period than has previously been demonstrated by culture tests.

Clinical observations. Six patients, one receiving placebo and five receiving famciclovir, had prodrome only and never developed skin lesions. HSV DNA could be detected in three of them (one on placebo and two on famciclovir) only by PCR amplification and then Southern blot hybridization. Ethidium bromide staining of the PCR products was negative, as were culture tests, thereby indicating that these three patients experienced very low levels of virus shedding. Of the other three patients, all receiving famciclovir, one individual was positive by PCR and virus growth, and two patients presented with prodrome only but were negative for HSV both by PCR plus hybridization and by virus culture. An additional five patients treated with famciclovir had negative PCR results and negative virus cultures, yet they developed typical herpetic skin lesions during the course of the study. Patients in this group developed genital papules and vesicles during days 1 to 4 of the recurrence, with rapid healing afterward. The presence of PCR inhibitors was ruled out after spiking HSV DNA into the extracts of clinical samples (data not shown).

Assessment of treatment effects. We assessed the effectiveness of famciclovir treatment at various dosages using either number of days with positive virus culture or days with a pos-

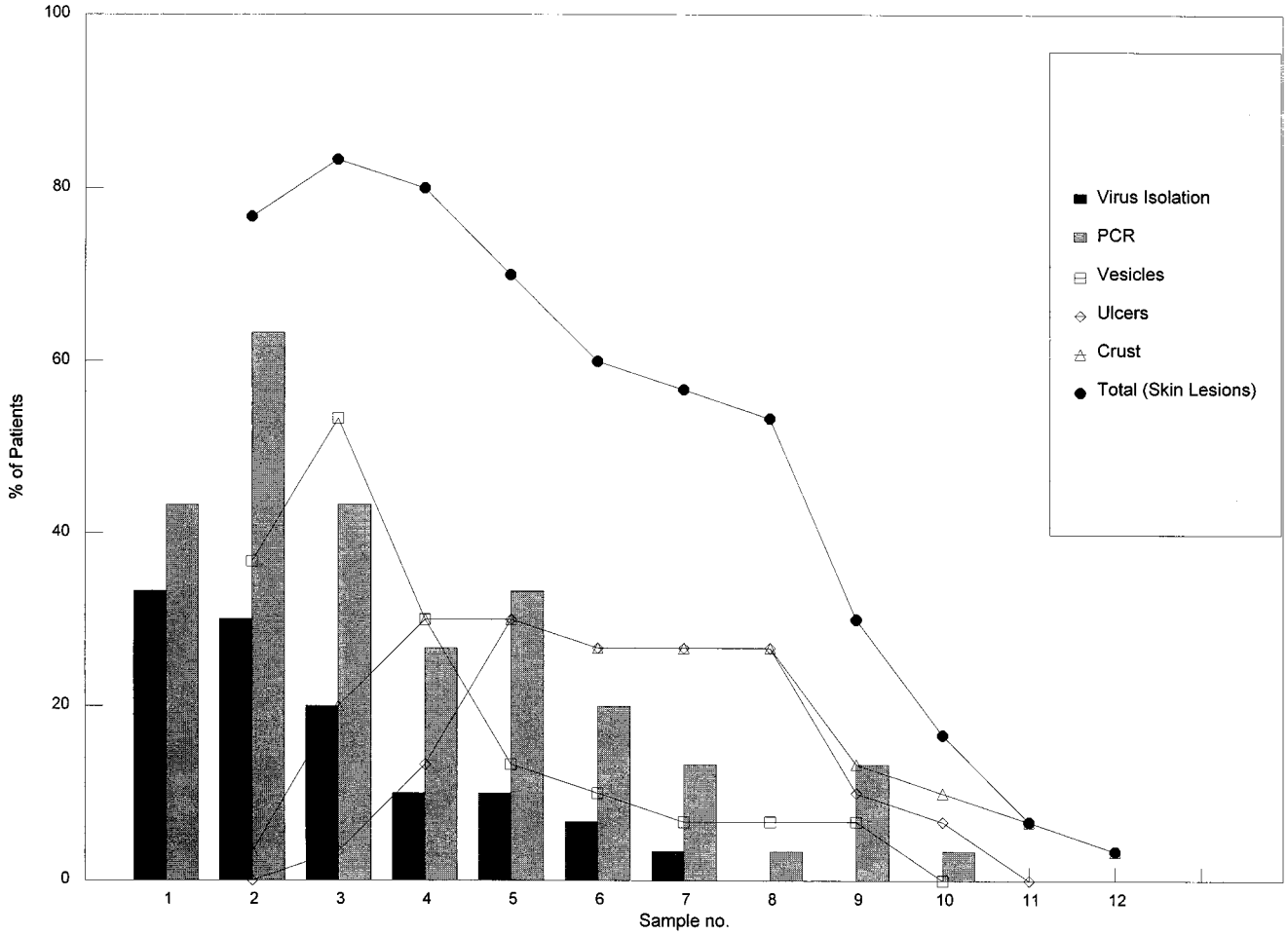

FIG. 3. Skin lesions, virus isolation, and DNA shedding in 30 patients with a recurrence of genital HSV-2 treated with famciclovir. Samples were collected as described in the legend to Fig. 2 from patients treated with 125, 250, or 500 mg of famciclovir twice daily. The total number of patients with skin lesions at each time point is shown. The bars represent virus isolation in tissue culture or viral DNA detection after PCR and then Southern blot hybridization with a labelled probe.

itive DNA PCR test result as the outcome. We performed separate one-way analyses of variance for each dependent variable (our data met the usual assumptions underlying analysis of variance: variance homogeneity and normally distributed data). The independent variable was treatment (four groups; placebo and famciclovir at 125, 250, and 500 mg). When virus culture was the outcome, a borderline significant overall F value was observed ($F_{3,36} = 2.56$; $P = 0.081$). Post hoc pairwise comparisons between group means by the least-significant-difference test indicated that the placebo group had a higher number of days with a positive test result than the group of subjects treated with 250 mg of famciclovir ($P < 0.05$). No other group means differed significantly.

The same analysis, but with the DNA PCR test result as the outcome, had highly significant results ($F_{3,36} = 8.36$; $P = 0.0002$). Pairwise comparison of group means by the least-significant-difference test found significant differences in the number of days with a positive test result between the placebo group and the group receiving famciclovir at 125 mg, the placebo group and the group receiving famciclovir at 250 mg, and the placebo group and the group receiving famciclovir at 500 mg ($P < 0.05$ for all comparisons). There were no significant differences between the famciclovir groups ($P > 0.10$ for all comparisons).

DISCUSSION

The concomitant appearance of detectable infectious virus or viral DNA and genital lesions was examined in patients with a recurrence of genital herpes. On the basis of this correlation, DNA amplification by PCR was found to be more sensitive as a diagnostic test than the detection of infectious virus in tissue culture, consistent with the results of others (11, 13). PCR alone and then visualization of the products with ethidium bromide allowed the detection of 10^{-4} $TCID_{50}$/ml for HSV-2. The additional use of Southern blot hybridization following PCR increased the sensitivity of the assay an additional 10^2, allowing the detection of 10^{-6} $TCID_{50}$/ml for HSV-2. The difference in sensitivity between the detection of infectious virus or DNA may have been due to a high concentration of noninfectious viral particles or free viral DNA in the stock cultures. Although the PCR cannot distinguish between infectious or noninfectious virus, this increased sensitivity was of clear clinical relevance in that it allowed the detection of HSV DNA in samples that were otherwise negative by culture and ethidium bromide staining of the PCR products. A total of six patients (28 new samples; Table 1), for whom all other tests were negative, were diagnosed by HSV DNA hybridization. The improved sensitivity of this method of detection allows for

a more precise description of the natural history of herpetic lesions and a more accurate assessment of the efficacies of antiviral drugs.

In the present study, we made use of the enhanced sensitivity of the PCR in detecting HSV to follow the natural history of genital herpetic recurrences in patients treated with placebo and the changes resulting from therapy with oral famciclovir. In both cases, the highest incidence of patients with genital skin lesions and of virus isolation was observed in the first 36 h following the onset of symptoms. The highest rates of detection of HSV or viral DNA were in samples taken from vesicles or ulcers. Antiviral therapy initiated at the onset of the recurrence was effective in decreasing infectious virus and viral DNA shedding.

It should be noted that in some of our samples, herpetic lesions were not associated with detectable virus or viral DNA. It is possible that in such cases the levels of DNA in the lesions were below the levels of detection of the PCR and that the viral mRNA and proteins present in the infected cells, in the absence of viral DNA replication, mediated the appearance and the maintenance of the lesions. Inhibitors of PCR may sometimes be present in clinical samples, but they are usually eliminated by the DNA extraction procedure (20). There was no evidence of PCR inhibitors in the genital samples analyzed in the present study.

Another subset of patients had prodromic symptoms coincident with the detection of viral DNA but did not develop skin lesions. Five of these patients were treated with famciclovir, suggesting that viral DNA inhibitors used early at the onset of the prodrome may sometimes abort recurrences of genital herpes. The same clinical presentation, however, also occurred in one patient treated with placebo, which suggests that recurrences may also be aborted naturally by the host's immune system.

Viral DNA replication and the release of infectious virions may be the main event immediately prior to the appearance of skin lesions in genital herpes infections. Famciclovir, the oral form of penciclovir, effectively blocks viral DNA synthesis by inhibiting the viral DNA polymerase by the triphosphate form of the drug found in high concentrations in HSV-infected cells (2, 8, 23). Earnshaw et al. (8) reported the rapid formation and a long half-life of the active triphosphated form of penciclovir in HSV-infected cells, thus allowing for a decreased frequency of administration of the antiviral drug. Famciclovir reduced the number of patients experiencing skin lesions following the onset of the prodrome and decreased by more than one-third the number of patients positive for viral DNA present at the site of lesion within the first 24 h. Nevertheless, famciclovir therapy did not prevent the appearance of recurrences in most patients, since the virus was already replicating in the area of the lesion at the time that the treatment was started. Administered at the onset of the prodrome, however, this antiviral agent appears to inhibit further virus replication, shorten the evolution of the lesions, and decrease virus shedding.

The complementary use of PCR and virus culture allowed us to study the natural history of herpetic lesions and associated HSV shedding during a recurrence of genital herpes. The use of PCR and then Southern blot hybridization significantly improved the sensitivity of our diagnostic procedures. This increased sensitivity should prove valuable in assessing patients at risk of recurrences of genital herpes, particularly among women in the later stages of pregnancy. PCR allows screening for herpesvirus to be carried out with accuracy (5), thereby increasing the chances of identifying newborns at risk of infection. In addition, this procedure may be able to detect viral DNA before the development of clinical disease. It may also be able to characterize an abortive recurrence of genital herpes. With PCR automation and decreased cost, this test may be a very useful clinical tool in the management of patients with herpesvirus infections.

The present study suggests that the DNA PCR method is also more sensitive than virus culture or clinical observations, such as features of skin lesions or lesion duration, in evaluating treatment effectiveness. Statistically significant differences were found by the DNA PCR method between each group treated with famciclovir and the placebo group, while a significant treatment effect was observed only in the 250-mg famciclovir group (compared with the placebo group) by using virus culture as the outcome. This has important financial ramifications for designing clinical trials, since a more precise outcome measure will necessitate a smaller sample size for a trial.

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REFERENCES

1. **Arvin, A. M., P. A. Hensleigh, C. G. Prober, D. S. Au, L. L. Yasukawa, A. E. Wittek, P. E. Palumbo, S. G. Paryani, and A. S. Yeager.** 1986. Failure of antepartum maternal cultures to predict the infant's risk of exposure to herpes simplex virus at delivery. N. Engl. J. Med. **315:**796–800.
2. **Bacon, T. H., and R. F. Schinazi.** 1993. An overview of the further evaluation of penciclovir against herpes simplex virus and varicella-zoster virus in cell culture highlighting contrasts with acyclovir. Antiviral Chem. Chemother. **4**(Suppl. 1)**:**25–36.
3. **Brown, Z. A., J. Benedetti, R. Ashley, et al.** 1991. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. N. Engl. J. Med. **324:**1247–1252.
4. **Coleman, R. M., L. Pereira, P. D. Bailey, D. Dondero, C. Wickliffe, and A. J. Nahmias.** 1983. Determination of herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **18:**287–291.
5. **Cone, R. W., A. C. Hobson, Z. Brown, R. Ashley, S. Berry, C. Winter, and L. Corey.** 1994. Frequent detection of genital herpes simplex virus DNA by polymerase chain reaction among pregnant women. JAMA **272:**792–796.
6. **Cone, R. W., A. C. Hobson, J. Palmer, M. Remington, and L. Corey.** 1991. Extended duration of herpes simplex virus DNA in genital lesions detected by the polymerase chain reaction. J. Infect. Dis. **164:**757–760.
7. **Cone, R. W., P. Swenson, A. Hobson, M. Remington, and L. Corey.** 1993. Herpes simplex virus detection from genital lesions: a comparative study using antigen detection (HerpChek) and culture. J. Clin. Microbiol. **31:**1774–1776.
8. **Earnshaw, D. L., T. Bacon, S. Darlison, K. Edmonds, R. Perkins, and A. Vre Hodge.** 1992. Mode of antiviral action of penciclovir in MRC-5 cells infected with herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus. Antimicrob. Agents Chemother. **36:**2747–2757.
9. **Gleaves, C. A., D. Wilson, A. Wold, and T. Smith.** 1985. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h postinoculation. J. Clin. Microbiol. **21:**29–32.
10. **Goto, T., Y. Yamaguchi, M. Hashido, H. Yoshikawa, and T. Kawana.** 1993. Diagnosis of genital herpes by polymerase chain reaction amplification. Microbiol. Immunol. **37:**987–990.
11. **Hardy, D. A., A. N. Arvin, L. L. Yasukawa, R. N. Bronzan, D. M. Lewinsohn, P. A. Hensleigh, and C. G. Prober.** 1990. Use of polymerase chain reaction for successful identification of asymptomatic genital infection with herpes simplex virus. J. Infect. Dis. **162:**1031–1035.
12. **Hsiung, G. D.** 1982. Diagnostic virology, p. 5–29. Yale University Press, New Haven, Conn.
13. **Kimura, H., M. Shibata, K. Kuzushima, K. Nishikawa, Y. Nishikawa, and T. Morishima.** 1990. Detection and direct typing of herpes simplex virus by polymerase chain reaction. Med. Microbiol. Immunol. **179:**177–184.
14. **Ou, C. Y., S. Kwok, S. W. Mitchell, D. H. Mak, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science **239:**295–297.
15. **Prober, C. G., P. A. Hensleigh, F. D. Boucher, L. L. Yasukawa, D. S. Au, and**

A. M. Arvin. 1988. Use of routine viral cultures at delivery to identify neonates exposed to herpes simplex virus. N. Engl. J. Med. **318:**887–891.
16. **Rogers, B. B., S. L. Josephson, and P. J. Sweeney.** 1992. Polymerase chain reaction amplification of herpes simplex virus DNA from clinical samples. Obstet. Gynecol. **79:**464–469.
17. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Shibata, D., W. J. Martin, M. D. Appleman, D. M. Causey, J. M. Leedom, and N. Arnheim.** 1988. Detection of cytomegalovirus DNA in peripheral blood of patients infected with human immunodeficiency virus. J. Infect. Dis. **158:**1185–1192.
20. **Shibata, D. K., N. Arnheim, and W. J. Martin.** 1988. Detection of human papilloma virus in paraffin-embedded tissue using the polymerase chain reaction. J. Exp. Med. **167:**225–230.
21. **Sullender, W. M., L. L. Yasukawa, M. Schwartz, L. Pereira, P. A. Hensleigh, C. G. Prober, and A. M. Arvin.** 1988. Type-specific antibodies to herpes simplex type 2 (HSV-2) glycoprotein G in pregnant women, infants exposed to maternal HSV-2 infection at delivery, and infants with neonatal herpes. J. Infect. Dis. **157:**164–171.
22. **Vere Hodge, R. A., and Y.-C. Chang.** 1993. The mode of action of penciclovir. Antiviral Chem. Chemother. **4**(Suppl. 1)**:**13–24.
23. **Vere Hodge, R. A., and R. Perkins.** 1989. Mode of action of 9-(4-hydroxy-methylbut-1)-guanine (BRL 39123) against herpes simplex virus in MRC-5 cells. Antimicrob. Agents Chemother. **33:**223–229.
24. **Whitley, R. J., L. Corey, A. Arvin, F. D. Lakeman, C. V. Sumaya, P. F. Wright, L. M. Dunkle, S. J. Soong, and A. J. Nahmias.** 1988. Changing presentation of herpes simplex virus infection in neonates. J. Infect. Dis. **158:**109–116.
25. **Whitley, R. J., A. Yeager, P. Kartus, Y. Bryson, J. D. Connor, A. J. Nahmias, and S. J. Soong.** 1983. Neonatal herpes simplex virus infection: follow-up evaluation of vidarabine therapy. Pediatrics **72:**778–785.