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Correlation between Response to Acyclovir and Foscarnet Therapy and In Vitro Susceptibility Result for Isolates of Herpes Simplex Virus from Human Immunodeficiency Virus-Infected Patients

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In vitro susceptibility testing of herpes simplex virus (HSV) isolates will play an increasingly important role in guiding the clinical management of immunocompromised hosts who have lesions that are poorly responsive to therapy with standard antiviral agents. We assessed the correlation between the in vitro susceptibility result using a plaque reduction assay in Vero cells and the response to antiviral therapy with acyclovir or foscarnet for 243 clinical isolates of HSV collected from 115 human immunodeficiency virus-infected patients. The in vitro results and clinical responses were highly associated for both acyclovir and foscarnet ($P < 0.001$ and $P < 0.001$, respectively). The predictive values of a susceptible result (50% effective concentrations, <2 μg/ml for acyclovir and <100 μg/ml for foscarnet) for complete healing of lesions were 62% for acyclovir and 82% for foscarnet; the predictive values of a resistant result for failure to heal were 95% for acyclovir and 88% for foscarnet. Thus, in vitro testing has clinical utility in guiding therapy, although the 1 to 2 weeks required to derive a definitive result by the plaque reduction assay is a major limitation.

The efficacy of acyclovir for the treatment and suppression of anogenital herpes simplex virus (HSV) infections has been established in placebo-controlled trials (4, 5, 7). Despite estimates of the prevalence of acyclovir-resistant mutants within mucocutaneous herpetic lesions which range from 0 to 6.2% in untreated immunocompetent patients (1, 3), cutaneous healing has generally been unimpeded in these hosts. In immunocompromised patients, however, particularly those with human immunodeficiency virus (HIV) infection, acyclovir-resistant strains of HSV may ultimately predominate within a clinical lesion, resulting in deeply ulcerating, progressive infections despite acyclovir therapy (2, 9). Sporadic reports of resistance to foscarnet therapy in immunocompromised patients receiving this drug for the treatment of acyclovir-resistant HSV lesions have recently appeared as well (8, 10). We sought to investigate the relationship between the clinical response to therapy with acyclovir or foscarnet and the in vitro susceptibility test result for clinical HSV isolates collected from HIV-infected patients.

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MATERIALS AND METHODS

Clinical HSV isolates were referred to our laboratory for antiviral susceptibility testing by clinicians in the United States. We did not recruit specific specimens, and no criteria were imposed to qualify a specimen as acceptable for testing other than our ability to propagate the virus in cell culture, using MRC-5 cells, upon receipt. Susceptibilities to acyclovir and foscarnet were tested by the plaque reduction assay as described previously (10). Monolayers of Vero cells containing minimum essential medium and 10% fetal calf serum (FCS) were infected with 30 to 60 PFU of virus per 100 μl. We added sterile powders of acyclovir (Burroughs Wellcome Company, Research Triangle Park, N.C.) and foscarnet (Astra Pharmaceuticals Inc., Westborough, Mass.) that had been solubilized in sterile water and diluted with 2% FCS to the following desired concentrations: 0.1, 0.2, 0.8, 3.1, 12.5, 25, and 50 μg/ml for acyclovir and 12.5, 25, 50, and 100 μg/ml for foscarnet. Plaques were overlaid with methylcellulose, stained with a crystal violet solution, and counted manually. The number of plaques present at a given drug concentration was derived from the average of three replicates. The percentage of remaining plaques at various drug concentrations against the percentage of remaining plaques in the wells representing drug-free controls was plotted on semilogarithmic probit paper, and the concentrations of antiviral drug required to inhibit plaque formation by 50% (EC_{50}) and 90% (EC_{90}) were calculated graphically. We defined acyclovir resistance as an EC_{50} of ≥2 μg/ml and resistance to foscarnet as an EC_{50} of ≥100 μg/ml (10). Acyclovir-susceptible (VL3-S), acyclovir-resistant (VL5-R), foscarnet-susceptible (KOS), and foscarnet-resistant (PAA^r5) control strains were tested along with each clinical isolate.

Clinical information was obtained from the referring physician by written request by using a standardized questionnaire (10). We requested data regarding the duration of the patient's herpetic outbreak, the patient's absolute CD4 cell count, the route and duration of antiviral therapies administered to the patient, and the patient's response to therapy. A complete response to therapy was defined as full healing (i.e., reepithelialization) of all lesions; in partial responders, enlargement of the lesion ceased, but full healing did not occur. Therapeutic

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failure was defined as continued enlargement of the lesion despite antiviral therapy.

Continuous values are described by using either the mean with the standard deviation or the median and range. Statistical comparisons were made by the Wilcoxon signed-rank test and logistic regression. Logarithmic transformation was used to more accurately calculate peak and nadir EC_{50}s from frequency histograms. The utility of in vitro susceptibility testing as a predictor of the clinical response to antiviral therapy was assessed by using sensitivity (the proportion of patients whose lesions healed completely and who had a susceptible result), specificity (the proportion of patients whose lesions failed to heal and who had a resistant result), positive predictive value (the probability of complete healing in patients with a susceptible result), and negative predictive value (the probability of failing to heal in patients with a resistant result).

RESULTS

Between January 1989 and January 1992, we received a total of 362 HSV isolates from 201 patients with requests for antiviral susceptibility testing. This report focuses on the subset of 243 isolates from 115 HIV-infected patients for whom we have clinical information regarding their responses to antiviral therapy. Eighteen patients (16%) were women. The CD4 cell count at the time of collection of the specimen for virus culture was available for 81 patients; the median CD4 cell count was 16/mm^3 (range, 0 to 840/mm^3).

Predictors of response to antiviral therapy. The HSV type of 227 (92%) isolates was determined; the majority (86%) were HSV type 2. Virus type was not associated with clinical response to therapy with either acyclovir or foscarnet in this sample ($P = 0.2$ and $P = 0.3$, respectively). Of 221 instances in which the location of the clinical lesion was known, 54% were perirectal, 12% were genital, 25% were orofacial, and 9% were elsewhere. Patients with perirectal lesions were significantly more likely than those with lesions in other locations to fail to respond to acyclovir therapy (16 versus 43%; $P = 0.006$), and patients with orofacial lesions were more likely to heal (44 versus 21%; $P = 0.02$). The location of the lesion was not significantly associated with the response to foscarnet therapy. The absolute CD4 cell count was not associated with the response to either acyclovir ($P = 0.1$) or foscarnet ($P = 0.3$), although the analysis of this variable was limited by the fact that data were available for only 69 and 44 patients, respectively. We found no association of gender and response to therapy with either acyclovir ($P = 0.2$) or foscarnet ($P = 0.3$).

In vitro susceptibility patterns. A bimodal distribution of EC_{50}s of acyclovir was found (Fig. 1A). The two peaks in Fig. 1A represent 87 acyclovir-susceptible isolates (mean EC_{50}, 0.7 ± 0.4 μg/ml) and 156 acyclovir-resistant isolates (mean EC_{50}, 28.7 ± 32.7 μg/ml). The nadir between the two peaks was 3.3 μg/ml.

The EC_{50}s of foscarnet approximated a unimodal distribution (Fig. 1B). The mean EC_{50} for 227 foscarnet-susceptible isolates was 25.7 ± 16.9 μg/ml. However, the curve was skewed to the right by the presence of 15 foscarnet-resistant isolates (EC_{50}s, 100 to 260 μg/ml), representing 6% of all isolates tested. Six of these 15 isolates (40%) were cross-resistant to foscarnet and acyclovir.

Clinical correlation of in vitro result: Acyclovir. Information regarding the response of the HSV lesion to acyclovir therapy was available for 115 patients (Table 1). Of 10 patients who had a partial response to acyclovir, 8 were infected with isolates that had in vitro susceptibility to acyclovir (EC_{50} range, 0.2 to 1.1 μg/ml). Testing of serial isolates from three patients in this category revealed multiple instances of phenotypic variation to and from acyclovir resistance over time. Serial isolates from the two partial responders whose isolates showed resistance to acyclovir in vitro were unavailable for testing (EC_{50}s, 11 and 3.3 μg/ml, respectively). We were unable to identify differences in the route or dose of acyclovir therapy, the size of the lesion, or absolute CD4 cell count that distinguished partial responders from those with complete or absent responses.

Complete healing was reported in 24 of 39 (62%) patients from whom the recovered virus was susceptible to acyclovir (Table 1). Seven patients (18%) failed to respond to acyclovir therapy, despite the in vitro susceptibilities of their infecting isolates. Acyclovir-resistant isolates were recovered from two

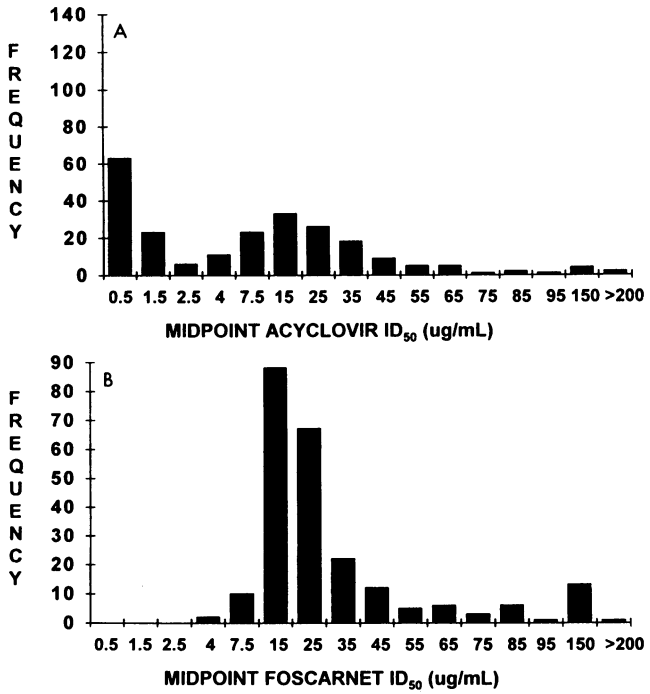

FIG. 1. Distribution of in vitro susceptibility values. (A) Acyclovir. (B) Foscarnet. The EC_{50} (ID_{50}) represents the concentration of antiviral drug required to inhibit plaque formation by 50%.

TABLE 1. Association of in vitro susceptibility to acyclovir and clinical response to acyclovir therapy

In vitro susceptibility result[a]	Response to antiviral therapy (no. of patients)[b]			
	Complete healing	Partial healing	Failure to heal	Total
Susceptible (EC_{50}, <2 μg/ml)	24	8	7	39
Resistant (EC_{50}, ≥2 μg/ml)	2	2	72	76
Total	26	10	79	115

[a] The EC_{50} was derived by the plaque reduction assay with Vero cells. It represents the concentration of acyclovir required to inhibit plaque formation by 50%.

[b] A complete response signifies full healing, a partial response signifies the absence of enlargement of the lesion without full healing, and failure denotes enlargement of the lesion despite antiviral therapy.

of these seven patients 2 to 3 months earlier, suggesting the presence of a population of acyclovir-resistant strains within the clinical lesion that had not yet reestablished predominance. For a third patient, however, each of two perirectal isolates obtained over a time span of nearly 6 months showed susceptibility to acyclovir, such that there was no explanation for the lack of response.

A lack of response to acyclovir was reported in 72 of 76 (95%) patients whose isolates were resistant to acyclovir in vitro (Table 1). In two patients, complete healing of the lesions occurred following therapy with acyclovir, despite the in vitro resistance of the infecting isolates (EC_{50}s, 15 and 15 μg/ml, respectively). For one of the two patients in this category, an isolate recovered from the same site 3 months later again showed resistance to acyclovir; no serial isolates from the other patient were available for testing.

The sensitivity of the plaque reduction assay test result for a complete clinical response to acyclovir was therefore 92% (24 of 26 patients), and the specificity was 91% (72 of 79 patients). The ability of a susceptible result by the plaque reduction assay to predict a complete clinical response to acyclovir therapy (i.e., the positive predictive value) was 24 of 39 patients (62%), and the negative predictive value of a result indicating resistance predicting a failure to heal was 72 of 76 patients (95%).

Acyclovir EC_{50}s were significantly lower for isolates from patients with a complete response to acyclovir (median, 0.7 μg/ml; range, 0.1 to 15 μg/ml) than for isolates from patients who failed to respond to acyclovir therapy (median, 17 μg/ml; range, 0.18 to 207.2 μg/ml) ($P < 0.001$; Fig. 2). Also, for isolates from patients in whom the response to acyclovir was partial, the EC_{50}s were significantly lower (median, 1.0 μg/ml; range, 0.2 to 11 μg/ml) than those for isolates from patients with a lack of response ($P < 0.001$), although median EC_{50}s for isolates from partial responders did not differ significantly from those with for isolates from complete responders ($P = 0.5$).

We found a strong association of the acyclovir EC_{50} result with the clinical response to acyclovir therapy when the analysis was stratified by our chosen threshold for resistance (EC_{50}, ≥2 μg/ml [$P < 0.001$]). Variation of EC_{50} threshold for resistance to values of 1.5, 2.5, and 3.0 μg/ml or to the natural breakpoint of the frequency distribution (EC_{50}, 3.3 μg/ml) did not result in a superior statistical correlation with response to acyclovir therapy when compared with that of our chosen threshold (i.e., the χ^2 value was lower and the P value did not decrease [data not shown]).

The threshold EC_{90} for resistance to acyclovir is undefined. Analysis of the association of the clinical response to acyclovir with the EC_{90}, when stratified either by the natural breakpoint for isolates when visualized graphically (EC_{90}, 20 μg/ml) or by the median (EC_{90}, 44 μg/ml), resulted in lower absolute χ^2 values and no improvement in statistical significance when compared with the use of the EC_{50}. We were unable to find a breakpoint which offered a superior statistical association with clinical response compared with that of the EC_{50}.

Clinical correlation of in vitro result: foscarnet. The response to therapy with foscarnet was highly associated with the in vitro susceptibility result ($P < 0.001$; Table 2). Thirteen of the 75 patients (17%) for whom data regarding their clinical response to therapy was available manifested a partial response to therapy with foscarnet: isolates from 12 patients showed susceptibility to foscarnet in vitro (range of EC_{50}s, 9 to 70 μg/ml). Serial isolates tested from 3 of these 12 patients showed persistent susceptibility to foscarnet; two subsequent isolates collected from a fourth patient 2 and 3 weeks later showed resistance in vitro (EC_{50}s, 119 and 130 μg/ml, respectively), and the patient failed to respond to therapy with that drug (10).

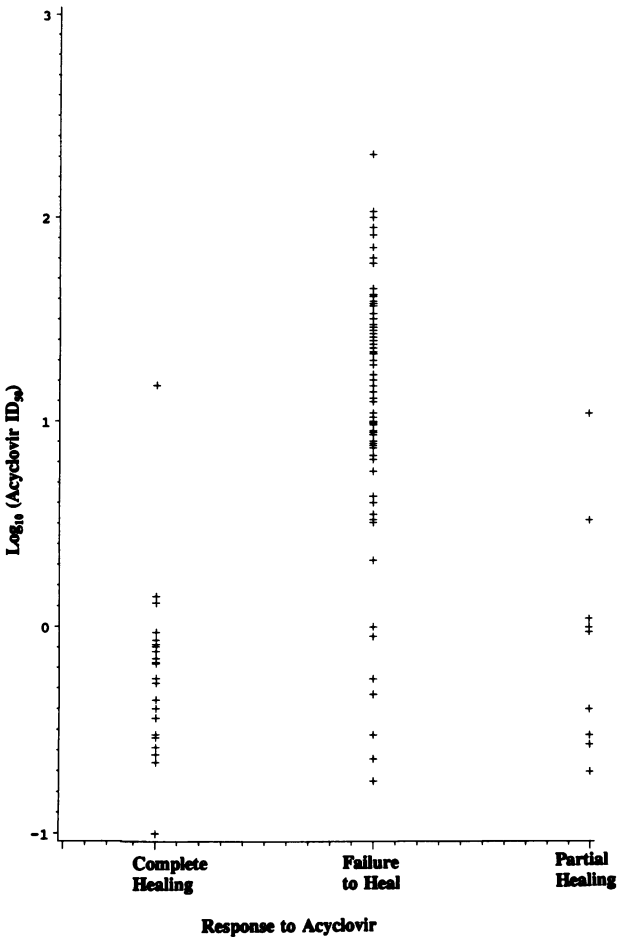

FIG. 2. Association of acyclovir EC_{50}s (ID_{50}) and clinical response to acyclovir therapy. Twenty-six patients healed completely in response to acyclovir (EC_{50} range, 0.1 to 15 μg/ml; median, 0.7 μg/ml), 79 patients failed to heal (EC_{50}, range, 0.18 to 207.2 μg/ml; median, 17 μg/ml), and 10 patients had partial healing (EC_{50} range, 0.2 to 11 μg/ml; median, 1 μg/ml). Note that EC_{50}s are represented on a $\log_{10}$ scale (each plus sign indicates an individual patient).

Complete healing was reported for 55 of 67 (82%) patients whose isolates were demonstrated to be susceptible in vitro (Table 2). The foscarnet EC_{50} was <100 μg/ml for isolates from patients who failed to heal in response to foscarnet therapy. Conversely, for isolates from seven of eight patients who failed to respond to foscarnet therapy, foscarnet EC_{50}s were ≥100 μg/ml. The sensitivity of susceptibility testing for a complete clinical response to foscarnet was therefore 100% (55 of 55 patients), and the specificity was 100% (7 of 7 patients). The positive predictive value for a susceptible in vitro test result was 82% (55 of 67 patients) for a complete clinical response, while a result indicating resistance predicted a failure of the clinical response to foscarnet in 88% (7 of 8) of patients.

EC_{50}s were significantly lower for isolates collected from patients who healed completely in response to foscarnet therapy (median, 17 μg/ml; range, 7.3 to 40 μg/ml) than those for isolates from patients who failed to heal (median, 119 μg/ml; range, 100 to 260 μg/ml) ($P < 0.001$; Fig. 3) as well as

TABLE 2. Association of in vitro susceptibility to foscarnet and clinical response to foscarnet therapy

In vitro susceptibility result[a]	Response to antiviral therapy (no. of patients)[b]			
	Complete healing	Partial healing	Failure to heal	Total
Susceptible (EC_{50}, <100 μg/ml)	55	12	0	67
Resistant (EC_{50}, ≥100 μg/ml)	0	1	7	8
Total	55	13	7	75

[a] The EC_{50} was derived by the plaque reduction assay with Vero cells. It represents the concentration of foscarnet required to inhibit plaque formation by 50%.

[b] A complete response signifies full healing, a partial response signifies the absence of enlargement of the lesion without full healing, and failure denotes enlargement of the lesion despite antiviral therapy.

from those in whom the response to foscarnet therapy was partial (median, 24 μg/ml; range, 9 to 119 μg/ml) rather than absent ($P = 0.001$). Interestingly, for those with a partial rather than complete response, the median EC_{50}s for the isolates were somewhat higher, although this did not reach statistical significance ($P = 0.09$).

Critical evaluation of our chosen threshold definition for foscarnet resistance (EC_{50}, ≥100 μg/ml) was restricted by the small number of isolates for which the EC_{50}s were high and by limited information regarding the correlative clinical responses in these patients.

DISCUSSION

In the study described here, testing of the in vitro susceptibilities of HSV type 1 and HSV type 2 to acyclovir and foscarnet was highly predictive of the therapeutic responses to those agents in HIV-infected patients. The lack of ability to predict with 100% accuracy complete healing or failure to heal by susceptibility values for acyclovir and foscarnet was primarily due to the presence of partial responders rather than to patients with a clinical response overtly disparate from that suggested by in vitro test results. Although we were unable to identify discrete clinical or virologic factors which would predict disparity between in vitro test results and clinical results, we speculate that one of three factors may be responsible. A heterogeneous virus population within clinical lesions composed of a mixture of susceptible and resistant strains, as proposed by previous investigators (6, 8), might account for misleading susceptibility test results, possibly because of a transition in a predominant strain that is ongoing at the time that the clinical lesion is sampled. Alternatively, it is conceivable that following the successful resolution of a lesion caused by an acyclovir-resistant HSV isolate, a latent acyclovir-susceptible isolate reactivates, as demonstrated in a previous clinical trial (9). This is supported by our finding in several patients of variation from the acyclovir-resistant to -susceptible phenotype when lesions from patients with recurrent episodes were sampled over time. Finally, a clinical response to antiviral therapy disparate from the in vitro result may reflect the interaction of host factors with virologic ones in determining the response to therapy.

The fact that median EC_{50}s were significantly higher for isolates from patients who failed to respond to either acyclovir or foscarnet therapy than for isolates from patients who healed completely, as well as the highly statistically significant association of the in vitro result with the clinical response to therapy

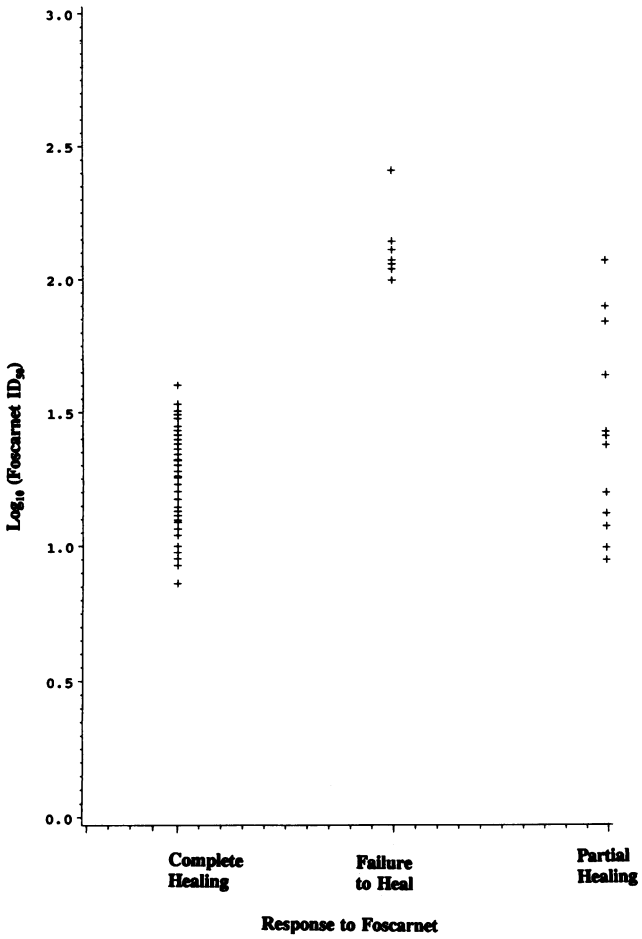

FIG. 3. Association of foscarnet EC_{50}s (ID_{50}s) and clinical response to foscarnet therapy. Fifty-five patients healed completely in response to foscarnet (EC_{50} range, 7.3 to 40 μg/ml; median, 17 μg/ml), 7 patients failed to heal (EC_{50} range, 100 to 260 μg/ml; median, 119 μg/ml), and 13 patients had partial healing (EC_{50} range, 9 to 119 μg/ml; median, 24 μg/ml). Note that EC_{50}s are represented on a $\log_{10}$ scale (each plus sign represents an individual patient).

with these agents, suggests that in vitro susceptibility testing has the potential to be of benefit to the clinician in guiding the choice of antiviral therapy. Our chosen thresholds defining resistance to acyclovir and foscarnet in vitro, i.e. ≥2 and ≥100 μg/ml, respectively, appeared to have clinical relevance. Nevertheless, the turnaround time of 1 to 2 weeks for performance of the plaque reduction assay once a clinical specimen is received limits the ability of the laboratory to assist in clinical decision making. Continued investigation of more rapid, reproducible, and clinically relevant in vitro assays is therefore warranted.

Several sources of potential bias in the present study should be considered. First, the results of in vitro susceptibility testing may vary according to the assay and cell type used and from laboratory to laboratory. Although we did not routinely retest isolates to confirm the EC_{50}s, we performed blinded retesting on a random sample of 39 isolates. Initial and repeat EC_{50}s of acyclovir had a correlation coefficient of 0.86 ($P < 0.001$), and paired foscarnet EC_{50}s had a correlation coefficient of 0.82 ($P < 0.001$), indicating an acceptable degree of reproducibility. Second, the likelihood that isolates were referred to us for

antiviral susceptibility testing only when a patient was failing to respond to therapy as expected might be construed as imposing a selection bias. Although it is clear that our sample of clinical HSV isolates was nonrandom, the representation of isolates both susceptible and resistant to acyclovir and foscarnet, as well as that of isolates from patients with complete and failed responses, allowed our analysis to be relatively broad.

Despite a previous suggestion that the EC_{90} result might constitute a superior predictor of clinical responsiveness to a given antiviral agent (11), we were unable to demonstrate an advantage of the EC_{90} result over the EC_{50} result for predicting the clinical response to either acyclovir or foscarnet therapy. In fact, since relatively few of the plaques were counted in the laboratory at the stage at which 90% were inhibited in growth, we feel that the EC_{50} is likely a more accurate and reproducible measure of drug susceptibility.

We conclude that in vitro susceptibility testing by the plaque reduction assay is a clinically relevant assay for testing the susceptibilities of HSV isolates to acyclovir and foscarnet. The disadvantages of the assay include its imperfect ability to predict a clinical response, its requirement for 1 to 2 weeks to yield a definitive result, and the need for an experienced technician. However, its close correlation to the clinical response to a given antiviral agent in the sample of patients analyzed ensures its utility in guiding therapy for HIV-infected patients in whom the response to therapy is other than that expected or in whom alternative therapies are needed.

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