ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1996, p. 2721–2726 Vol. 40, No. 12
0066-4804/96/$04.00+0

Effect of Foscarnet on Quantities of Cytomegalovirus and Human Immunodeficiency Virus in Blood of Persons with AIDS

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Received 22 May 1996/Returned for modification 23 July 1996/Accepted 18 September 1996

Four intravenous dosages of foscarnet given for 10 days were compared with no therapy in persons with AIDS who had asymptomatic cytomegalovirus (CMV) viremia. CMV viremia was quantitated by endpoint cell dilution microcultures, pp65 antigenemia assay, and measurement of CMV DNA in peripheral blood leukocytes by a quantitative-competitive PCR. Human immunodeficiency virus type 1 (HIV-1) viremia was quantitated by endpoint cell dilution microculture, serum p24 antigen assay, and PCR for HIV-1 RNA in plasma. Twenty-seven subjects who had received a median of 22 months of nucleoside antiretroviral therapy were enrolled. Twenty-two subjects received foscarnet, which was well tolerated and decreased the CMV burden, as reflected by all three indicator assays. During the 10 days of dosing, the level of CMV viremia, as measured by 50 percent tissue culture infective doses, decreased from 117.5 to 12.7 ($P = 0.001$), the amount of CMV DNA decreased from 20,328 copies to 622 copies per 150,000 leukocytes ($P = 0.02$), and the level of CMV pp65 antigenemia decreased from 14.9 to 1.6 positive peripheral blood mononuclear cells per 50,000 leukocytes ($P = 0.008$). A significant pharmacodynamic relationship was found between the peak foscarnet concentration and a decrease in the level of CMV antigenemia ($P < 0.05$). Foscarnet had no effect on quantitative HIV-1 microcultures during the 10 days of treatment, but the HIV-1 p24 antigen level in serum decreased significantly, from 454 to 305 pg/ml ($P = 0.01$). Also, a significant pharmacodynamic relationship was seen between plasma HIV-1 RNA concentrations and both peak foscarnet concentration ($P < 0.01$) and the area under the foscarnet time-concentration curve ($P < 0.05$). Reductions in the levels of CMV and HIV-1 viremia correlated quantitatively with systemic exposure to foscarnet, whereas control subjects actually experienced an increase in CMV and HIV-1 burdens. The dual antiviral activity of foscarnet shown in this trial encourages investigation of its use in combination with other antiretroviral therapies for persons with AIDS.

Foscarnet (Foscavir; Astra USA) has demonstrable in vitro and in vivo activities against cytomegalovirus (CMV) and human immunodeficiency virus type 1 (HIV-1), which makes it an attractive antiviral drug for individuals infected with HIV-1, especially those with low $CD4^+$ lymphocyte counts who are at risk for CMV disease (6, 12, 15, 31). The effect of foscarnet against CMV and HIV-1 might explain the survival benefit observed in persons with AIDS randomized to foscarnet versus ganciclovir for initial treatment of CMV retinitis, although both drugs had a similar effect on the progression of retinitis (28). Because this dual activity of foscarnet has not been explored quantitatively in a pharmacodynamic, controlled clinical trial, we used six different viral assays to measure changes in the levels of CMV and HIV-1 viremia associated with a 10-day exposure to four different dosages of foscarnet in persons with AIDS. Subjects with CMV viremia were chosen to participate in the study because we postulated that CMV viremia would precede tissue-invasive CMV disease, and control of viremia at the asymptomatic stage might delay or prevent CMV disease. The doses of foscarnet were selected to include a dose estimated to have no effect against CMV and HIV-1 and a maximum tolerated dose in order to establish quantitative relationships between foscarnet concentration and its antiviral effects. At the same time, the foscarnet susceptibilities of CMV and HIV-1 strains were monitored to learn if there was a correlation between relative susceptibility and the pharmacodynamic effects and also to determine if short-term therapy resulted in the emergence of foscarnet resistance.

(This study was presented in part at the 18th International Congress of Chemotherapy, Stockholm, Sweden, 1 July 1993; the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans La., 17 to 20 October 1993; and the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 17 to 20 September 1995.)

MATERIALS AND METHODS

Study design. This was a multicenter study performed at the University of Minnesota, Beth Israel Hospital/Harvard, and North Shore University Hospital/Cornell. The Minnesota site served as the central laboratory for quantitative virologic assays. The study was approved by the institutional review board at each center. Persons with AIDS and $CD4^+$ lymphocyte counts of $<200/ml^3$ who had CMV viremia but who had never manifested tissue-invasive CMV disease were eligible to participate. Viremia was documented by the isolation of CMV in conventional cultures within 4 weeks of enrollment. Previous antiretroviral therapy was allowed and was not altered during the trial. Additional enrollment criteria were as follows: age, ≥18 years; Karnofsky performance score, ≥60; and life expectancy, ≥6 months. Pregnant or lactating women were not enrolled. Other exclusions to participation were the presence of active CMV disease on screening examination, prior treatment of CMV disease with either foscarnet or ganciclovir, receipt of acyclovir within the past 4 weeks, and treatment with inhibitors of renal tubular excretion or loop diuretics.

Subjects were randomized to one of four different 10-day intravenous dosages

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of foscarnet (15 mg/kg of body weight every 8 h, 30 mg/kg every 8 h, 45 mg/kg every 12 h, and 90 mg/kg every 12 h) or no therapy. Foscarnet sodium (provided by Astra USA) was administered by an infusion pump through a central or peripheral venous line at a rate of 1 mg/kg/min. Normal saline was used as the hydration fluid. Foscarnet doses were initially adjusted according to the 24-h creatinine clearance measured at the baseline. Dose adjustments were based on the calculated creatinine clearance performed on each day of the study according to the recommendations provided by the manufacturer in the package insert. A rise in the serum creatinine level to 2.8 mg/dl or a drop in creatinine clearance to 50% or more from the baseline necessitated the discontinuation of foscarnet and withdrawal from the study.

Blood samples were collected at least daily except on Sundays and holidays for the first 10 days, and then approximately 1, 4, and 6 weeks later. The specific blood collection times for the pharmacologic assays are given below. Daily sampling during the first 10 days was done to monitor any early virologic response and to assess the interassay variability and biologic variability of the quantitative virologic tests. The CMV laboratory studies that were performed on all samples were quantitative microculture and pp65 antigenemia assay. The HIV-1 virology assays performed on all samples were serum p24 antigen assay and RNA PCR. The CMV quantitative-competitive PCR (QC-PCR) assay, HIV-1 quantitative microculture, and assays determining the susceptibilities of CMV and HIV-1 isolates to foscarnet were done at the baseline, day 10, and day 42. The methodologies for these assays are outlined below.

Pharmacologic studies. On day 1, blood samples were collected before and at the end of the first infusion of foscarnet and at 0.5, 1, 3, 5, and 7 h thereafter for participants receiving doses every 8 h. For those receiving doses every 12 h, an additional blood sample was obtained 10 h after the end of the infusion. On days 2, 4, 5, 7, and 10, one blood sample was obtained between 2 and 6 h after completion of the first infusion for that day. Plasma was separated from each blood sample by centrifugation and was stored at −20°C until it was analyzed. Urine was collected at the baseline and then in 2-h periods beginning with the first infusion on day 1 and continuing for the duration of the dosing interval (8 or 12 h) for the first dose only. The total volume of urine was recorded, and a 20-ml aliquot was stored at −20°C for measurement of the foscarnet concentration by ion-paired reversed-phase liquid chromatography (23).

Pharmacokinetic parameters for the disposition of foscarnet in plasma were determined by fitting a two-compartment model to the plasma concentration-time data by a Bayesian estimation strategy. The effect of foscarnet on the quantities of CMV and HIV-1 was explored with linear and maximal effect models. The foscarnet exposure variables examined included the 10-day cumulative area under the curve (AUC_C), peak and trough concentrations, and peak concentration/trough concentration ratios. Both a percent suppression and normalized area-under-the-curve (NAUC) approach were taken to explore foscarnet's effect on surrogate markers. The NAUC was calculated by dividing the AUC for a given marker by the denominator baseline value multiplied by time. In this regard, NAUC values greater than 1.0 indicate an increase in that parameter over the study period, values equal to 1.0 correlate with no change, and values less than 1.0 indicate a net decrease. All models were implemented with the ADAPT II package of programs (8).

CMV quantitative microcultures. Leukocytes (WBCs) were obtained from 3 to 5 ml of EDTA-treated blood by gradient centrifugation by using Polymorphoprep (Nycomed Pharma AS, Oslo, Norway) solution. After centrifugation, all leukocyte bands were transferred to a new tube, washed three times in phosphate-buffered saline (PBS), and resuspended in 2 ml of culture medium (minimal essential medium, 2% fetal bovine serum, and antibiotics). Five serial fivefold dilutions of 0.2 ml of the WBC suspensions (containing 1×10^6, 2×10^5, 4×10^4, 8×10^3, and 3.2×10^2 cells, respectively) were inoculated in duplicate into 24-well microculture plates containing confluent monolayers of human foreskin fibroblasts. After a 2-h absorption period, the inoculum was removed and 1 ml of culture medium was added to each well. The plates were incubated for 4 weeks and were observed under the microscope at 24 h and weekly thereafter for the appearance of a cytopathic effect. Wells showing a cytopathic effect were scored as positive. The results were expressed as 50% tissue culture infective doses ($TCID_{50}$s) per 10^6 WBCs.

CMV antigenemia assay. The CMV antigenemia assay was performed with a commercial kit (CMV-vue kit; INCSTAR Corp., Stillwater, Minn.) according to the manufacturer's instructions, with some modifications (11). WBCs were obtained from 3 to 5 ml of EDTA-treated blood by gradient centrifugation. Contaminating erythrocytes were lysed in an NH_4Cl solution for 4 min at room temperature. WBC fractions were washed three times in PBS, counted, and resuspended in culture medium. Aliquots of the WBC suspensions containing 50,000 cells were placed in duplicate wells of specially coated glass microscope slides. After incubation for 15 min at room temperature, the slides were fixed in acetone for 10 min, air dried, and incubated for 45 min at 37°C with a mixture of two murine monoclonal antibodies (INCSTAR Corp.) directed against the CMV protein pp65. This was followed by immunoperoxidase staining of the slides with a horseradish peroxidase-antimouse immunoglobulin G antibody. The slides were then observed under a light microscope for the presence of red-brown nuclear or perinuclear staining indicative of CMV infection. The average number of positive cells from two wells (expressed as the number of positive cells per 50,000 WBCs) was used to quantitate the level of CMV viremia.

CMV QC-PCR. Quantitation of CMV DNA in WBCs was performed as described by Boivin et al. (3). Briefly, a set of dilutions containing 5 to 50,000 copies of a plasmid including a portion of the CMV glycoprotein H (gH) sequence (external standard) was coamplified in the presence of 100 copies of another plasmid containing a modified sequence of the same CMV gene (internal standard). In addition, DNA obtained from the WBCs was also coamplified in the presence of 100 copies of the internal standard. One of the PCR primers was directly 5′ end labeled with a fluorescent dye to allow detection of the amplified products by measuring the amount of fluorescence emitted by each PCR fragment with an automated DNA sequencer and the Genescan 672 software (Applied Biosystems). This software is designed to measure the size, height, and area of specific DNA peaks corresponding to each specific amplified product. A standard curve was obtained by plotting the log of the external standard peak area/internal standard peak areas against the log of the copy number of the external control added to the reaction mixture. The number of copies of the CMV gH gene present in WBC samples was determined by interpolating the log of the ratio of amplified products (WBC samples/internal standard) into the standard curve. The results were expressed as the number of gH copies present per 150,000 WBCs.

CMV susceptibility to foscarnet. Foscarnet susceptibilities were determined by a DNA hybridization technique described previously (2). Blood culture isolates collected on day 1 before therapy were paired with isolates collected on study day 9 or 10, and the isolates were tested simultaneously. The concentrations of foscarnet used in the assay were 0, 23.3, 38.9, 64.8, 108, 180, 300, and 500 μM. The results were expressed as the concentration of foscarnet (in micromolar) producing 50 percent inhibition of the virus (IC_{50}).

Quantitative microcultures for HIV and serum p24 antigen assay. The AIDS Clinical Trials Group methodologies for quantitation of infectious HIV-1 in peripheral blood leukocytes and measurement of HIV-1 p24 antigen levels in serum were used (1).

Quantitative PCR for HIV RNA. Quantitation of HIV RNA in plasma fractions was performed by a PCR method (Amplicor HIV Monitor Assay; Roche Molecular Systems, Somerville, N.J.) with HIV-1 *gag* primers SK-431 (5′-biotin-TGCTATGTCAGTTCCCCTTGGTTCTCT-3′) and SK-462 (5′-biotin-AGTTGGAGGACATCAAGCAGCCATGCAAAT-3′) and an internal standard control (20). The analytical sensitivity of this PCR assay is 200 copies of HIV RNA per ml of plasma.

HIV susceptibility to foscarnet. Foscarnet IC_{50}s for HIV-1 isolates were performed by the AIDS Clinical Trials Group–U.S. Department of Defense consensus assay for zidovudine susceptibilities (16), which was modified by using as the viral input 100 μl of viral stock supernatant whose viral titer was not predetermined instead of a standardized input of 1,000 $TCID_{50}$s of viral stock. Blood culture isolates collected on day 1 before therapy were paired with isolates collected on study day 9 or 10, and the isolates were tested simultaneously.

Statistics. Differences between mean pre- and posttherapy quantitative viral measures were assessed by a nonparametric test for paired comparisons (Wilcoxon signed rank test). Results were based only on data for those subjects for whom quantifiable results from at least two assays were available, one of which was for a sample collected at the baseline or on day 1, before infusion of the study compound. Comparisons of positive and negative test results were done by the corrected chi-square test or the Fisher exact test (when the expected number was five or less). The comparisons between foscarnet recipients and control subjects were made by the Mann-Whitney U test. The multivariate regression analysis was initially completed to explore the relationships between foscarnet exposure variables and effect markers. These relationships were further explored by using the pharmacodynamic models mentioned above. The pharmacodynamic relationships between foscarnet exposure and effect were explored statistically by determining the correlation coefficient of the relationship. Two-tailed *P* values of ≤0.05 were considered to be significant.

RESULTS

Screening and demographics of participants. Between 1 May 1992 and 31 August 1993, 354 blood samples from 271 subjects were cultured for CMV viremia. Fifty-one (18.8%) of the volunteers screened were viremic, and 27 of them (25 men and 2 women) enrolled in the study. The median age of the participants was 37 years (mean age, 37.4 years; age range, 25 to 52 years). Their median $CD4^+$ lymphocyte count was 20 cells per ml^3 (mean, 27.7 cells per ml^3; range, 1 to 89 cells per ml^3). All participants had received antiretroviral nucleoside analogs as monotherapy (16 subjects) or in combination (11 subjects) for a median of 22 months (mean, 21.6 months; range, 2 to 51 months). Five (18.5%) of the 27 participants had discontinued their antiretroviral therapy a mean of 4 months (range, 1 to 9 months) prior to enrollment in the present study. Twenty-two subjects were randomized to one of four foscarnet dosages: six subjects each received 15 mg/kg every 8 h, 45

TABLE 1. Virologic assay results for CMV and HIV-1

Virus and quantitation method	Mean ± SD % change from baseline (no. of subjects)					
	Dosing every 8 h		Dosing every 12 h		All foscarnet recipients	Controls (n = 5)
	15 mg/kg (n = 4)	30 mg/kg (n = 4)	45 mg/kg (n = 5)	90 mg/kg (n = 4)		
CMV						
Microculture	−83.3 ± 22.6 (4)	−96.2 ± 7.5 (4)	−98.1 ± 2.7 (3)	−98.7 ± 2.3 (3)	−92.9 ± 13.0 (14)	55.4 ± 234 (4)
QC-PCR	−98.0 (1)	−96.6[a]	−96.3 ± 4.0 (3)	−71.2 ± 47.6 (4)	−85.4 ± 32.2 (9)[a]	383 ± 670 (3)
pp65 antigenemia assay	−86.1 ± 19.6 (2)	19.5 ± 138 (2)	−64.9 ± 6.3 (3)	−95 ± 7.1 (2)	−59.5 ± 68 (9)	−9.2 ± 102 (5)
HIV-1						
Microculture	55.1 ± 234 (4)	−40.5 ± 47 (4)	131.7 ± 250 (5)	80 ± 277 (3)	59.8 ± 207 (16)	504 ± 1078 (5)
p24 antigen assay	−32.3 ± 5.6 (2)	−48.5 ± 17.6 (3)	−25.3 ± 40.6 (4)	−58.5 ± 14 (2)	−38.6 ± 27.4 (11)[b]	7.1 ± 31.5 (3)
HIV RNA assay	−12.3 ± 21 (4)	0.5 ± 20 (4)	−21.3 ± 28 (4)	−12.9 ± 87 (4)	−11.5 ± 44 (16)[c]	53.4 ± 58 (4)

[a] Pooled data from the 30-mg/kg group were used because one of four patients in this group had increases of from 5 to 249 copies per 150,000 WBCs (4,880%); individual data cause skews in the distribution since the maximum possible decrease is 100%.
[b] $P < 0.04$ compared with controls (Mann-Whitney U test).
[c] $P < 0.05$ compared with controls (Mann-Whitney U test).

mg/kg every 12 h, and 90 mg/kg every 12 h, whereas four subjects received 30 mg/kg every 8 h. Five participants were randomized to the control (no drug) group.

Correlation of CMV viremia and CD4$^+$ lymphocyte count at screening. Complete screening data were collected only at the Minnesota site. Recent CD4$^+$ lymphocyte counts were available for 75 (74%) of the 102 subjects at the Minnesota site. The probability of having CMV viremia was highly correlated with the CD4$^+$ lymphocyte count. The proportions of viremic subjects by CD4$^+$ lymphocyte count strata were as follows: >100 cells per ml^3, 0 of 16 subjects (0%); 50 to 99 cells per ml^3, 5 of 24 subjects (21 percent); and <50 cells per ml^3, 19 of 35 subjects (54 percent). The differences in these proportions were statistically significant ($P < 0.0001$).

Adverse events. Serum creatinine concentrations did not change appreciably during the 10-day period of infusions. One subject required a dose reduction on the second day of the study, from 90 to 45 mg/kg, because of hypocalcemia (a decrease in total calcium from 8.4 to 7.8 mg/dl) and a prolonged QT interval on his electrocardiogram. He received oral calcium supplements for the remainder of the study, which he completed without further incident. There were two dose reductions for minor fluctuations in creatinine clearance. A patient in the 15-mg/kg group received 908 mg for 13 doses, 806 mg for 12 doses, and 732 mg for his last 5 doses. A patient in the 90-mg/kg group received 7,074 mg for eight doses, 6,480 mg for eight doses, and 6,225 mg for his last four doses.

Quantitative effects of foscarnet on CMV and HIV-1 viremia. Quantitative CMV data were analyzed only for subjects at the Minnesota site, because it was found that overnight shipment of samples resulted in a substantial loss of detectable virus. After beginning the treatment phase of this protocol, 22 subjects at the Minnesota site provided a total of 411 blood samples for virologic studies. Study assignments for these 22 subjects were as follows: 15 mg/kg every 8 h, four subjects; 30 mg/kg every 8 h, four subjects; 45 mg/kg every 12 h, five subjects; 90 mg/kg every 12 h, four subjects; and control group, five subjects. Table 1 provides data on the mean percent change from the baseline for all six virologic assays and gives standard deviations showing wide fluctuations among subjects. The following data use absolute values for comparisons made between the four foscarnet treatment groups combined and the control group, because the small number of subjects in each treatment group made most comparisons between individual groups and control subjects not statistically significant. Foscarnet reduced the level of CMV viremia, as reflected by all three indicator assays. Data from all time points were examined, but since the antiviral effect was most evident when baseline values were compared with the results obtained on study day 10, these are the data presented. Quantitative CMV microculture results decreased from a mean pretherapy level of 117.5 ± 290 (standard deviation [SD]) $TCID_{50}$s per 10^6 WBCs to 12.7 ± 44.1 (SD) $TCID_{50}$s per 10^6 WBCs on day 10 ($P = 0.001$); QC-PCR studies showed that CMV DNA levels decreased from a mean pretherapy level of 20,328 ± 62,444 (SD) copies per 150,000 leukocytes to 622 ± 1,891 (SD) copies on day 10 ($P = 0.02$); and the pp65 antigenemia assay showed that the levels decreased from a mean pretherapy level of 14.9 ± 26.7 (SD) positive cells per 50,000 blood leukocytes to 1.6 ± 3.6 (SD) positive cells on day 10 ($P = 0.008$). Significant pharmacodynamic relationships were observed between the decrease in the level of pp65 antigenemia and variables of foscarnet exposure, such as peak concentration (Fig. 1) and AUC ($P < 0.05$) (data

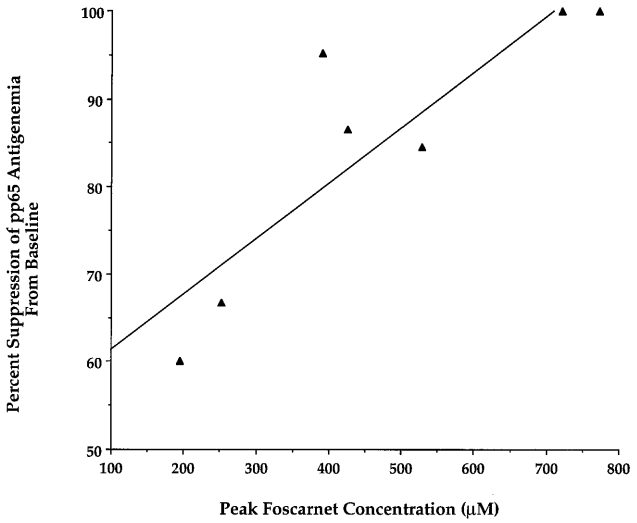

FIG. 1. Relationship between peak foscarnet concentration and decrease in level of CMV pp65 antigenemia ($P < 0.05$). Each datum point is the percent change from the baseline for an individual patient, and data are pooled across all dosage groups. For each patient there had to be at least two measurable points, including a baseline value, for the patient's data to be considered in this analysis.

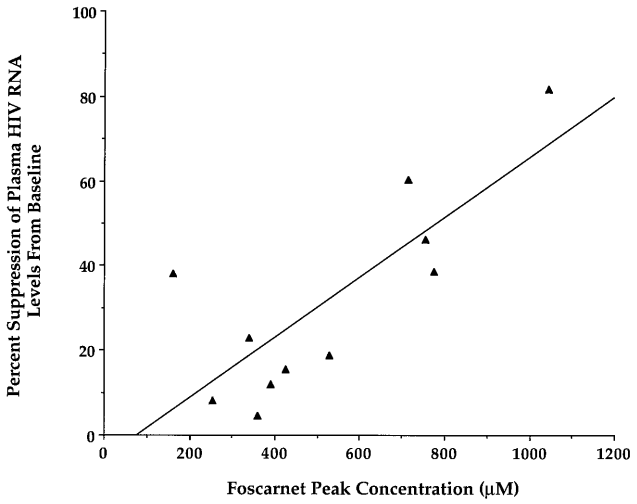

FIG. 2. Relationship between peak foscarnet concentration and decrease in plasma HIV-1 RNA concentration ($P < 0.01$). Each datum point is the percent change from the baseline for an individual patient, and data are pooled across all dosage groups. For each patient there had to be at least two measurable points, including a baseline value, for the patient's data to be considered in this analysis.

not shown). The foscarnet dosage corresponding to a 50% decrease in the pp65 antigenemia level was approximately 45 mg/kg every 12 h. A significant relationship was found by using the NAUC between the CMV pp65 antigen level and the foscarnet AUC_C ($r^2 = 0.702$; $P < 0.01$).

Quantitative HIV-1 data only for the 22 subjects at the Minnesota site were used in order for the analysis to be consistent with the CMV analysis. Foscarnet had no effect on quantitative HIV-1 microcultures during the 10 days of treatment. However, serum HIV-1 p24 antigen levels decreased significantly during the same period (mean pretherapy value of 454 ± 486 [SD] pg/ml versus 305 ± 375 [SD] pg/ml on day 10 [$P = 0.01$]). The plasma HIV-1 RNA level decreased from a mean pretherapy level of 460,657 ± 392,694 (SD) to 395,098 ± 294,619 (SD) copies per ml on day 10 for all dose groups. The 15-mg/kg dose group experienced a 15% decrease in plasma HIV-1 RNA levels, whereas the 90-mg/kg group had a 57% decrease. A significant relationship ($P < 0.01$) was apparent between the percent suppression of plasma HIV-1 RNA during the 10-day treatment period and the peak foscarnet concentration (Fig. 2). The HIV-1 p24 antigen level also decreased in response to foscarnet by using a linear model and percent suppression approach. The foscarnet AUC_C was significantly correlated with percent suppression of p24 antigen ($r^2 = 0.507$; $P < 0.05$), as were mean peak foscarnet concentrations ($r^2 = 0.404$; $P < 0.05$). Foscarnet AUC_C and peak foscarnet concentrations were also significantly correlated with the percent suppression of quantitative HIV-1 RNA (AUC_C $r^2 = 0.448$; $P < 0.05$ [peak $r^2 = 0.623$; $P < 0.01$]). A 50% reduction in plasma HIV-1 RNA levels and serum p24 antigen concentration corresponded to foscarnet dosages of 65 and 50 mg/kg every 12 h, respectively.

When quantitative virologic data were normalized for baseline CMV and HIV-1 load, the antiviral effect of foscarnet was seen regardless of the viral burden at the time of enrollment. In contrast to the 22 foscarnet recipients, the 5 control subjects had increases in their CMV or HIV-1 burdens, especially as reflected by CMV QC-PCR and HIV-1 microculture (Table 1).

Susceptibilities to foscarnet. No changes in the susceptibilities of the CMV or HIV-1 strains to foscarnet were observed by comparing isolates collected on day 1 with those collected on day 10. The median foscarnet IC_{50}s were 151 μM for day 1 CMV isolates and 155 μM for day 10 CMV isolates. The median foscarnet IC_{50}s for HIV-1 isolates from all 27 subjects were 43.7 μM for day 1 HIV-1 isolates and 37.6 μM for day 10 HIV-1 isolates. There were no differences in the foscarnet susceptibilities of the CMV and HIV-1 strains from foscarnet recipients compared with those from the control subjects. It was not possible to relate pharmacodynamic effects to baseline in vitro susceptibility data.

Pharmacokinetics. Foscarnet pharmacokinetic data were available for 21 patients. The respective mean peak and trough foscarnet concentrations for each dose group were as follows: 15-mg/kg group, 263 and 12 μM; 30-mg/kg group, 374 and 22 μM; 45-mg/kg group, 560 and 14 μM; and 90-mg/kg group, 832 and 30 μM. The volume of distribution at steady-state was 0.48 liter/kg, the elimination half-life was 6.0 h, and the total body clearance was 0.09 liter/kg/h. The mean AUCs from day 1 of therapy for each dose group were as follows: 15-mg/kg group, 1,309 μM · h; 30-mg/kg group, 2,462 μM · h; 45-mg/kg group, 3,003 μM · h; and 90-mg/kg group, 5,732 μM · h.

Clinical outcome. As expected during the short 10-day treatment period, there were no appreciable changes in $CD4^+$ lymphocyte counts for any of the subjects. For 25 subjects, physicians and patients provided follow-up data; 15 of the subjects died (3 of 5 in the control group and 12 of 20 in the foscarnet-treated group) from complications of HIV-1 disease a median of 264 days after enrollment. Ten participants were alive when they were last contacted, a median of 398 days after enrollment. Fourteen (56%) of 25 subjects (2 of 5 control subjects and 12 of 20 foscarnet-treated participants) developed CMV disease a median of 146 days after enrollment (mean, 139 days; range, 4 to 368 days). The median survival time after the diagnosis of CMV disease was 171 days (mean, 217 days; range, 74 to 459 days).

DISCUSSION

Foscarnet given intravenously for 10 days reduced the amount of CMV and HIV-1 in the blood of persons with AIDS who had already received nucleoside antiretroviral drugs for a median of 22 months. This reduction in the quantity of both viruses occurred with neither appreciable toxicity nor a change in the susceptibilities of the CMV or HIV-1 strains to foscarnet. Because the quantity of viremia has been related to progression of both viral diseases, the dual antiviral effect of foscarnet on both CMV and HIV-1 that was shown in our study, if sustainable, would likely result in clinical benefit. In the case of CMV, its presence and quantity in the blood predicts the development and progression of CMV disease in persons with AIDS (4, 10, 14, 24–27). In the case of HIV-1, the level of viremia and the ability of antiretroviral agents to lower that level are associated with improved outcomes. Persistently low levels of HIV-1 RNA in plasma are linked to long-term nonprogression to AIDS, whereas high levels in plasma are associated with a more rapid progression (5, 19, 22). A recent study found that the reduction in plasma HIV-1 RNA levels during the first 6 months of zidovudine therapy accounted for the majority of the benefit observed with that drug (21).

Even at the lowest dosage of foscarnet that we studied (15 mg/kg every 8 h) there was an effect on both CMV and HIV-1, but the larger doses produced a greater reduction in the quantities of both viruses. The larger doses did not cause appreciable toxicity in this study, but we realize that the duration of administration was shorter than that usually required for the induction of remission of CMV retinitis. Our study confirms

that current foscarnet dosing guidelines (90 to 120 mg/kg/day) are sufficient to inhibit the replication of CMV. The results suggest that larger doses or a longer period of therapy, however, are necessary to achieve a maximal antiretroviral effect.

The reduction in plasma HIV-1 RNA levels seen with foscarnet represented an absolute decrease of 0.6 log, which is less than the 1.0- to 2.0-log reduction reported for protease inhibitors (7). However, our subjects had received nucleoside antiretroviral therapy for nearly 2 years. In terms of its anti-HIV-1 activity, foscarnet is a nonnucleoside reverse transcriptase inhibitor. Prior use of nucleosides has been shown to reduce the quantitative anti-HIV-1 effect of nonnucleoside reverse transcriptase inhibitors (9). Therefore, it is reasonable to suggest that the effect of foscarnet on HIV-1 shown in this trial may have been blunted and that its maximum potential to reduce the HIV-1 viral burden would best be explored in nucleoside-naive individuals.

The relative lack of effect on HIV-1 microcultures (no reduction except in the 90-mg/kg dose group, as indicated in Table 1) is most likely because the microculture method stimulates the virions to grow in WBCs and provides some time for them to do so. Since foscarnet is virostatic, latent virions will have an opportunity to proliferate in the microcultures, thus masking the anti-HIV-1 effect of foscarnet. In contrast, both the serum p24 antigen and HIV RNA PCR assays directly measure the quantity of virus present in the original specimen.

In designing this study, we hypothesized that CMV viremia presages tissue-invasive CMV disease, and its reduction or ablation would result in a clinical benefit. We found that the probability of developing CMV disease in HIV-1-infected patients within a year after the documentation of CMV viremia was 56% (14 of 25 subjects), with a median time from the detection of CMV viremia to the diagnosis of CMV disease of 146 days. However, CMV viremia was not invariably followed by CMV disease, since 11 (44%) of our 25 subjects never developed tissue-invasive CMV disease after an average follow-up of 13 months. Therefore, CMV viremia should not be used as an absolute indicator for CMV prophylaxis in persons with HIV-1 infection or AIDS. The median survival time of our subjects after the diagnosis of CMV disease was 171 days, which is nearly identical to the median survival time of 173 days found after the diagnosis of CMV disease in a multicenter observational cohort study of persons with AIDS (13).

We thought that asymptomatic CMV viremia might occur in subjects whose $CD4^+$ lymphocyte counts were between 100 and 200 cells per ml^3, but we found no such instance. The relatively low rate of CMV viremia even in our subjects with $CD4^+$ lymphocyte counts of between 50 and 100 cells per ml^3 indicates that it is most practical to screen persons with <50 cells per ml^3 when CMV viremia is a prerequisite for inclusion in clinical trials.

Our study demonstrated a pharmacodynamic effect of foscarnet on HIV-1. This antiretroviral activity of foscarnet may explain the survival advantage in patients who received it in a randomized trial of foscarnet versus ganciclovir for the initial treatment of CMV retinitis (28). Although the findings from a subsequent retreatment trial by the same investigators appeared to contradict their previous findings, the two study populations were not comparable by the investigators' own admission (30). In fact, a survival advantage for those receiving foscarnet was apparent in the second study: for subjects who had received ganciclovir prior to enrollment in the retreatment protocol, the mortality rate was higher than that for those who had previously received foscarnet (relative risk, 1.44; 95% confidence interval, 1.00 to 2.09; $P = 0.05$).

Additional evidence now substantiates the direct effect of foscarnet on HIV-1. This includes the finding of a quantitative reduction in HIV-1 RNA levels during treatment of herpesvirus diseases (17) and the description of specific changes in HIV-1 reverse transcriptase, which render the mutants resistant to foscarnet (18). The development of resistance mutations is synonymous with specific antiviral activity. However, in a recent study of CMV retinitis in AIDS patients, both foscarnet and ganciclovir reduced serum HIV-1 p24 antigen concentrations (29). This suggested to the investigators that the anti-HIV-1 effect of foscarnet could have been an indirect result of reduced CMV replication. To conclusively prove an in vivo effect of foscarnet on HIV-1, it would have to be studied in individuals who are infected with HIV-1 but who are CMV naive.

The therapeutic benefit of an antiretroviral drug has been predicted from its initial effect on plasma HIV-1 RNA levels (21). In our study, blood samples were collected daily during the 10-day treatment period, and thus we were able to quantitate changes in CMV and HIV-1 loads immediately after the institution of foscarnet infusions. A novel finding was the additional anti-HIV-1 effect seen within 10 days of introducing foscarnet to individuals who had been on long-term antiretroviral therapy. This suggests that foscarnet might be considered a candidate drug for combination antiretroviral therapy in persons with advanced HIV-1 disease who are willing to contend with the inconvenience of receiving lengthy intravenous therapy.

ACKNOWLEDGMENTS

Carol A. Olson, Don Bennett, Nancy V. Reed, Nigel Rulewski, M. Colin Jordan, and Kathleen Brandt contributed ideas and effort to this study.

This study was supported in part by grant MO1-RR00400 from the National Center for Research Resources, grants AI27661 and AI33835 from the National Institutes of Health, and grants from Astra USA and the Minnesota Medical Foundation.

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