ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1994, p. 38–44
0066-4804/94/$04.00+0

Vol. 38, No. 1

Effect of Foscarnet Induction Treatment on Quantitation of Human Cytomegalovirus (HCMV) DNA in Peripheral Blood Polymorphonuclear Leukocytes and Aqueous Humor of AIDS Patients with HCMV Retinitis

GIUSEPPE GERNA,[1]* FAUSTO BALDANTI,[1] ANTONELLA SARASINI,[1] MILENA FURIONE,[1] ELENA PERCIVALLE,[1] M. GRAZIA REVELLO,[1] DONATO ZIPETO,[2] DAVIDE ZELLA,[2] AND THE ITALIAN FOSCARNET STUDY GROUP†

Virus Laboratory, Institute of Infectious Diseases, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico S. Matteo,[1] and Istituto di Genetica, Biochimica ed Evoluzionistica, Consiglio Nazionale delle Ricerche,[2] 27100 Pavia, Italy

Received 22 July 1993/Returned for modification 20 September 1993/Accepted 25 October 1993

The aim of this study was to investigate peripheral blood polymorphonuclear leukocytes and, whenever possible, aqueous humor from 65 AIDS patients with ophthalmoscopically diagnosed human cytomegalovirus (HCMV) retinitis to determine (i) whether patients consistently carry viral DNA and (ii) to what extent foscarnet induction treatment decreases viral DNA levels. HCMV DNA was quantified by PCR using densitometric analysis of hybridization products obtained from external standards and a standard curve from which the number of genome equivalents of test samples, normalized by using an internal amplification control, was interpolated. Results showed that 56 of 65 patients (86.1%) were positive for HCMV DNA prior to induction treatment. Of 41 of the 56 patients (73.2%) whose blood had become DNA negative after induction, only 5 had a high viral load (>5,000 genome equivalents per 2 × 10^5 polymorphonuclear leukocytes) prior to induction, whereas as many as 13 of the 15 (26.8%) patients remaining DNA positive after induction had a high viral load prior to induction. Finally, of the nine patients (13.8%) with DNA-negative blood prior to induction treatment, three were shifted to foscarnet from ganciclovir, while six were erroneously enrolled in the study. Pre- and postinduction aqueous humor samples were obtained from 12 patients; all of these were DNA positive prior to induction, whereas after induction, 4 became negative, 6 showed a marked decrease in viral DNA, and 2 had nearly stable low DNA levels. In conclusion, PCR is a valuable tool for etiologic diagnosis and monitoring of HCMV retinitis treatment in AIDS patients. HCMV DNA is consistently present in the blood and aqueous humor of all patients with HCMV retinitis. Foscarnet induction treatment is highly effective in suppressing or reducing DNA levels in both blood leukocytes and aqueous humor.

Induction regimens of both foscarnet and ganciclovir have been shown to be highly effective in halting progression of human cytomegalovirus (HCMV) retinitis in patients with AIDS (2, 4, 10–15, 17, 21). However, chronic maintenance therapy is required to forestall the progression of disease following induction (4, 12, 13, 16, 19).

In previous studies, the primary endpoint was the time (weeks) to progression of retinitis, while secondary endpoints included changes in visual acuity, HCMV shedding, human immunodeficiency virus p24 antigen levels, and total CD4 T lymphocyte counts (16). In the present study, the main purpose was to determine the effects of foscarnet induction treatment (IT) on HCMV DNA levels quantitated

* Corresponding author. Virus Laboratory, Institute of Infectious Diseases, University of Pavia, IRCCS Policlinico S. Matteo, 27100 Pavia, Italy. Phone: 39-382-502634. Fax: 39-382-423320.

† The following are the participating centers and the contributing investigators of the Italian Foscarnet Study Group. (i) Infectious Disease centers—Istituto di Malattie Infettive Università, Brescia: G. P. Carosi and F. Castelli; Istituto di Malattie Infettive Università, Pavia: R. Minoli and P. Grossi; Istituto di Malattie Infettive Università, Milan: M. Moroni, A. D'Arminio, and P. Coppin; Divisione Malattie Infettive Ospedale di Circolo, Lecco: A. Orani and D. Bolis; Ospedale S. Raffaele, Milan: A. Lazzarin and N. Gianotti; Divisione Malattie Infettive, Presidio Ospedaliero Cremonese, Cremona: G. Carnevale and A. Pan; Divisione Malattie Infettive Ospedale di Circolo, Busto Arsizio, Varese: F. Suter and G. Pellegata; Divisione Malattie Infettive Ospedale di Circolo, Varese: G. P. Fiori and C. Zeroli; Istituto Malattie Infettive Università, Bologna: F. Chiodo and O. Coronado; Divisione di Malattie Infettive Ospedali Riuniti, Parma: F. Fiaccadori and G. Magnani; Divisione Malattie Infettive, Ospedale Civile, Piacenza: F. Alberici and P. Viale; Divisione Malattie Infettive, Ospedale Niguarda, Ca' Granda, Milan: F. Giannelli and L. Gaggese; Divisione Malattie Infettive, Ospedale Civile, Padua: P. Cadrobbi and R. Scaggiante; Divisione Malattie Infettive, Ospedale C. Poma, Mantua: G. P. Cadeo and P. Costa; Divisione Malattie Infettive, Arcispedale S. Maria Nuova, Reggio Emilia: S. Gafà and M. G. Giudici. (ii) Ophthalmology centers—Istituto di Clinica Oculistica Università, Brescia: L. Ambrosoli; Istituto di Clinica Oculistica Università, Pavia: E. Antoniazzi and F. Trimarchi; Istituto di Clinica Oculistica Università, Milan: G. Bertoni, M. Blini, and S. De Angelis; Divisione Oculistica, Ospedale di Circolo, Lecco: E. Lanza; Clinica Oculistica, Ospedale S. Raffaele, Milan: A. Vergani; Divisione di Oculistica, Presidio Ospedaliero Cremonese, Cremona: M. Carrai; Divisione di Oculistica, Ospedale di Circolo, Busto Arsizio, Varese: A. Cazzola; Divisione di Oculistica, Ospedale di Circolo, Varese: L. Cerri and F. De Maria; Clinica Oculistica II, Policlinico S. Orsola, Bologna: L. Fontana; Clinica Oculistica, Ospedali Riuniti, Parma: A. Pescatori and G. Bontempelli; Clinica Oculistica, Ospedale Civile, Padua: A. Secchi, B. Turrini, and M. S. Tognon; Divisione di Oculistica, Ospedale C. Poma, Mantua: F. Laudando; Divisione di Oculistica, Arcispedale S. Maria Nuova, Reggio Emilia: A. Zotti.

by PCR in both blood leukocytes and, whenever available, aqueous humor. In parallel, tests for quantitation of HCMV antigenemia (7) and viremia (8) were performed. The results indicated that foscarnet is highly effective in suppressing or decreasing viral DNA levels in both blood and, to a slightly lesser degree, aqueous humor. However, the standard 3-week induction period may not be sufficient to clear HCMV DNA from blood and aqueous humor, particularly when the preinduction viral DNA load is high.

MATERIALS AND METHODS

Patients and samples. Patients at least 18 years of age with AIDS and HCMV retinitis were eligible for this study. The diagnosis of HCMV retinitis was based on the presence of characteristic white fluffy or granular retinal infiltrates with or without associated hemorrhages. Ophthalmologic eligibility criteria were determined by one or more ophthalmologists at each of 15 different institutions. Additional inclusion criteria were creatinine levels in serum of <2.0 mg/dl; hemoglobin value of ≥8.0 g/dl; a Karnofsky score of ≥50; absence of concurrent treatment with nephrotoxic drugs or drugs inhibiting renal tubular secretion; absence of corneal, lens, or vitreous opacification; and absence of concurrent treatment with ddI, ddC, or any experimental drug for AIDS treatment other than zidovudine (AZT).

Witnessed verbal informed consent was obtained, and eligible patients (designated by PFA number) were randomly assigned to receive a total of 3 weeks of intravenous foscarnet at a dosage of 60 mg per kg of body weight every 8 h administered in saline at a constant rate over 1 h (TID) or 90 mg per kg of body weight every 12 h at a constant rate over 2 h (BID). Fundus photographs were taken the day before initiation and the day following termination of IT. After completion of the study, entry fundus photographs were blindly reviewed by two ophthalmologists in the absence of any clinical and virologic information on the effectiveness of foscarnet IT. After IT, patients were discharged from the hospital and received single daily 2-h infusions of foscarnet as maintenance therapy at a dose of 90 mg/kg diluted to a total volume of 750 ml in saline. However, follow-up of patients during maintenance treatment was not part of the study.

Blood samples from all patients were collected just before the first drug infusion and on the day following the last administration. Peripheral blood polymorphonuclear leukocytes (PMNL) were used for determination and quantitation of HCMV antigenemia (7), viremia (8), and DNAemia in blood PMNL (leukoDNAemia), as described below. In addition, whenever possible, aqueous humor samples were collected before and after IT and examined for viral DNA quantitation only.

Antiviral susceptibility assay. Chemosensitivity testing of HCMV strains was performed using an immediate-early (IE) antigen plaque reduction assay recently developed in the laboratory and based on early detection of viral plaques 96 h postinfection by a monoclonal antibody to the major IE protein p72 (6).

HCMV DNA quantitation. For quantitation of viral DNA in PMNL and aqueous humor, two recombinant DNA molecules were constructed: one, referred to as pCM, was obtained by cloning the same HCMV IE1 gene region amplified by PCR for viral DNA detection in clinical samples (9); the other one, referred to as pAC, was obtained by cloning a recombinant DNA molecule flanked by the target sequences of primers used for HCMV IE1 gene amplification (22). pAC and pCM amplification products were identified by difference in electrophoretic mobility and by hybridization with specific probes. In coamplification assays, pAC and pCM showed the same amplification kinetics. Thus, the efficiency of amplification of the two molecules was comparable (22).

HCMV DNA quantification was achieved for blood by using an aliquot of 2×10^5 PMNL and for aqueous humor by using a 5-μl volume. In both types of samples, DNA was extracted by a rapid method based on the lysing and nuclease-inactivating properties of the chaotropic agent guanidinium thiocyanate and the nucleic acid-binding properties of silica particles (1). In each PCR test, serial dilutions of pCM containing 50,000, 5,000, 500, 50, 5, and 0.5 copies (external standards) were amplified in the presence of an amount of DNA equivalent to 2×10^5 HCMV DNA-negative PMNL for examining PMNL and in the presence of 5 μl of aqueous humor for testing aqueous humor samples. Both (standard and sample) amplification reactions were performed for 40 cycles in the presence of 100 pAC copies, which were used as an internal control of amplification to detect the presence of PCR inhibitors and to evaluate the PCR efficiency in each tube. Amplification products were visualized on 10% polyacrylamide gel following ethidium bromide staining. Hybridization was then performed by blotting 10 μl of amplification product in duplicate onto nylon membranes (MagnaGraph; Micron Separations, Inc., Westboro, Mass.) in a slot blot format and using probes specific for IE1 and pAC. Hybridization products were then detected by the chemiluminescence technique, and the signals obtained were submitted to densitometric analysis (22). The ratio pCM/pAC (100 copies) was used to plot a standard curve (Fig. 1). Quantitation of HCMV DNA in clinical samples was expressed as genome equivalents (GE) and was obtained from the standard curve by interpolating HCMV IE1/pAC (100 copies) hybridization product ratios.

Our PCR method allowed reproducible amplification of 15 GE per 2×10^5 PMNL or 15 GE per 5 μl of aqueous humor examined. Samples showing GE values of ≥15 were considered positive.

Statistical analysis. A *t* test was used (i) to compare levels of antigenemia, viremia, and DNAemia before and after foscarnet IT in patients remaining HCMV positive; (ii) to compare the two schedules of treatment (BID versus TID) in the evaluation of the effectiveness of antiviral treatment; and (iii) to evaluate the decrease in viral DNAs in both blood and aqueous humor after foscarnet IT. The chi-square test was used to evaluate the level of the viral load versus the persistence or disappearance of HCMV in blood PMNL, and, finally, the coefficient of correlation was determined to verify whether a correlation existed between levels of viral DNA in blood and those in aqueous humor.

RESULTS

Use of pAC as internal amplification control. Use of pAC (100 copies) as an internal control of amplification of clinical samples showed that 10 to 15% of the specimens contained PCR inhibitors. However, following DNA extraction by the silica procedure, the incidence of samples containing PCR inhibitors dropped to <1%. Thus, DNA extraction was used routinely throughout the study.

Patients. Of the 65 patients enrolled in the study, 56 (86.1%) were positive for HCMV in blood prior to IT by one or more assay methods (Tables 1 and 2). All of these 56 patients were positive for viral DNA by PCR, while 44

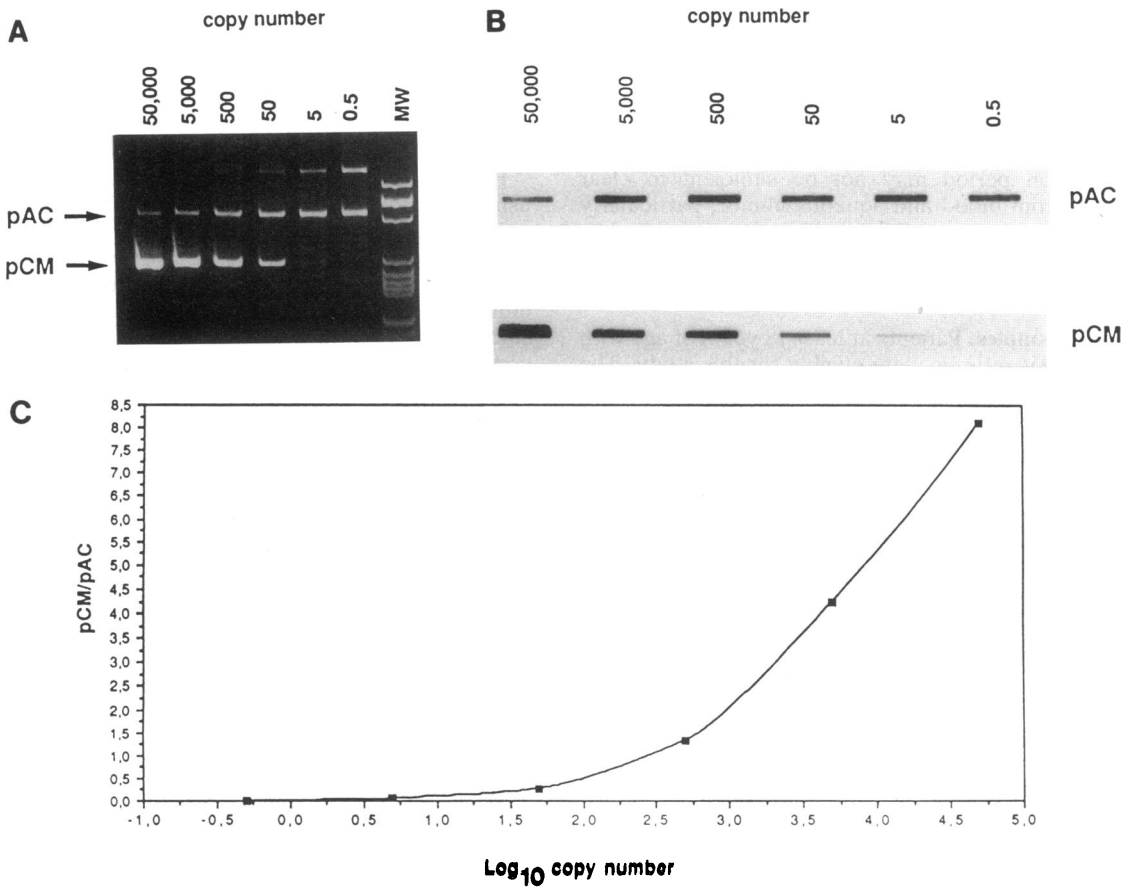

FIG. 1. Construction of a standard curve. (A) External standards (pCM copy number range, 5×10^4 to 0.5; pAC, 100 copies each sample) in 10% polyacrylamide gel following PCR amplification and ethidium bromide staining. MW, relative molecular weight markers. (B) Slot blot chemiluminescent hybridization of gel shown in panel A. (C) Standard curve obtained by densitometric analysis of slot blot hybridization signals.

(78.6%) were positive for pp65 antigenemia and 30 (53.6%) were positive for viremia. Thus, 12 patients (21.4%) were detected as positive only by PCR. All patients positive only by PCR had very low numbers of DNA copies (range, 15 to 140 GE).

Nine patients who were enrolled in the study with clinical diagnoses of HCMV retinitis were found negative for HCMV in blood prior to foscarnet IT by all assays. Retrospective examination of entry ophthalmoscopic photographs revealed that six of these nine patients were erroneously enrolled in the study and had non-HCMV-specific (cotton wool-like) ophthalmoscopic lesions. The remaining three patients were shifted to foscarnet therapy after 1 to 2 weeks of ganciclovir therapy because of drug toxicity detected a few days before enrollment in the study.

Effect of foscarnet IT on quantitative leukoDNAemia. Following IT, 41 of 56 patients (73.2%) had become negative for HCMV in blood by all assays, including PCR (Table 1). Of these 41 patients, prior to IT 29 were positive for pp65 antigenemia (mean antigenemia value, 43.6; range, 0 to 680), 15 for viremia (mean viremia value, 4.2; range, 0 to 40), and all 41 for leukoDNAemia (mean leukoDNAemia value,

TABLE 1. AIDS patients (n = 41) with retinitis positive for HCMV in blood prior to and negative for HCMV after IT with foscarnet

Blood sample taken	No. of patients with HCMV range in 2×10^5 PMNL by:										Virus isolation[d] (no. of patients)	
	pp65 antigenemia[a]			Viremia[b]			DNA[c]					
	0	1–10	>10	0	1–10	>10	<15	15–100	101–5,000	>5,000	Pos	Neg
Before IT	12	7	22	26	10	5	0	18	18	5	19	22
After IT	41	0	0	41	0	0	41	0	0	0	0	41

[a] pp65-positive PMNL (7).
[b] p72-positive fibroblasts in cell cultures 24 h after inoculation (8).
[c] HCMV GE.
[d] Positive (pos) or negative (neg) by conventional cell culture isolation technique (9).

TABLE 2. AIDS patients (n = 15) with retinitis positive for HCMV in blood before and after IT with foscarnet

Patient (treatment schedule)	Blood sample taken	HCMV quantitation in PMNL[a]			Virus isolation[b]
		pp65 Ag	Viremia	DNA	
PFA 3 (BID)	Before IT	420	39	≥50,000	Pos
	After IT	0	0	15	Neg
PFA 16 (TID)	Before IT	400	12	≥50,000	Pos
	After IT	0	0	15	Neg
PFA 19 (BID)	Before IT	≥1,000	400	≥50,000	Pos
	After IT	≥1,000	ND[c]	7,943	Pos
PFA 23 (BID)	Before IT	538	140	≥50,000	Pos
	After IT	0	0	501	Neg
PFA 34 (TID)	Before IT	276	40	28,183	Neg
	After IT	10	0	398	Neg
PFA 38 (TID)	Before IT	364	400	19,952	Pos
	After IT	4	1	15	Neg
PFA 39 (BID)	Before IT	110	28	35,481	Pos
	After IT	34	0	1,200	Neg
PFA 43 (TID)	Before IT	54	1	446	Pos
	After IT	1	0	20	Neg
PFA 44 (TID)	Before IT	≥1,000	120	≥50,000	Pos
	After IT	250	0	450	Neg
PFA 47 (BID)	Before IT	200	1	15,848	Pos
	After IT	1	0	20	Neg
PFA 56 (TID)	Before IT	71	39	1,122	Neg
	After IT	0	0	20	Neg
PFA 57 (TID)	Before IT	900	80	17,943	Pos
	After IT	0	0	35	Neg
PFA 75 (BID)	Before IT	295	14	≥50,000	Pos
	After IT	0	0	158	Neg
PFA 76 (BID)	Before IT	≥1,000	163	≥50,000	Pos
	After IT	75	0	125	Neg
PFA 81 (TID)	Before IT	130	12	11,584	Pos
	After IT	0	0	20	Neg

[a] See footnotes *a* to *c* for Table 1. Ag, antigenemia.
[b] Positive (pos) or negative (neg) by conventional cell culture isolation technique (9).
[c] ND, not done (sample not available).

2,449; range, 15 to 19,952 GE). In the group of 41 patients who became negative post-IT, only 5 (12.2%) had >5,000 GE (Table 1). In contrast, in the group of 15 patients (26.8%) who remained positive for HCMV after IT (Table 2), mean levels of pp65 antigenemia, viremia, and leukoDNAemia were 450.5 (range, 54 to ≥1,000), 99.3 (range, 1 to 400), and 32,037 (range, 446 to ≥50,000) GE, respectively, prior to IT, whereas mean levels were 91.7 (range, 0 to ≥1,000), 0.07 (range, 0 to 1), and 729 (range, 15 to 7,943) post-IT, respectively (t test pre-versus posttreatment, $P < 0.01$ for all three parameters). Thirteen of fifteen patients (86.6%) remaining positive post-IT had >5,000 GE pre-IT ($P < 0.01$ versus responders).

Overall, 36 of 38 patients (94.7%) with low viral load (<5,000 GE) became negative by all three assays, whereas only 5 of 18 (27.8%) with high viral load (>5,000 GE) became negative at the end of IT (chi-square test, $P < 0.001$). No statistically significant difference between the two treatment schedules (BID versus TID) was observed in the number of patients becoming negative for antigenemia, viremia, or DNAemia after IT.

Effect of foscarnet IT on viral DNA in aqueous humor. Pre- and posttreatment aqueous humor samples were available from 13 patients, whereas single pretreatment samples were taken from 3 additional patients (Table 3). Of these 16 patients examined by PCR of the aqueous humor, 15 were positive for HCMV DNA in both blood and aqueous humor before IT, while a single patient negative for HCMV in blood was also negative for the aqueous humor. This patient was one of the six who were not affected by HCMV retinitis and thus were erroneously enrolled in the study. Prior to IT, for the 12 HCMV-positive patients with sequential paired samples, the mean leukoDNAemia level was 10,605 (range, 15 to 35,481) GE, whereas in aqueous humor the mean DNA level was 10,774 (range, 172 to ≥50,000) GE. Following IT, the mean level of viral DNA in blood leukocytes was 91.0 (range, <15 to 1,200) GE and in aqueous humor it was 1,050 (range, <15 to 10,351) GE. A significant decrease of viral DNA in both blood (t test, $P < 0.01$) and aqueous humor ($P < 0.05$) was found. Of the 12 patients whose aqueous humor was examined following IT, 4 became negative for HCMV DNA, 6 showed a sharp decrease in viral DNA, and 2 (PFA 50 and 70) showed persistent low levels of DNA.

Analysis of the 12 patients with HCMV retinitis from whom paired blood and aqueous humor samples were available showed that (i) all patients positive for HCMV DNA in the eye before IT were also positive in blood; (ii) following IT, 4 (PFA 36, 59, 67, and 89) became negative for viral DNA in both blood and aqueous humor, 4 (PFA 38, 39, 56, and 57) remained positive for both sites, and 4 (PFA 20, 50, 70, and 86) remained positive in the eye while negative in blood; (iii) while in some patients the numbers of HCMV GE detected in blood and eye were grossly comparable, in other patients the amount of viral DNA was enormously greater at either site (correlation, $P > 0.05$) and this was true for both pre- and post-IT samples (Fig. 2). The two patients (PFA 50 and 70) who had no significant variation in the levels of viral

TABLE 3. Effect of foscarnet induction treatment on both PMNL and aqueous humor from 15 patients with HCMV retinitis

Patient (treatment schedule)	Blood and aqueous humor samples taken	HCMV quantitation[a]			
		PMNL			Aqueous humor
		pp65 Ag	Viremia	DNA	DNA
PFA 20 (TID)	Pre-IT	4	0	15	50,000
	Post-IT	0	0	<15	10,351
PFA 28 (TID)	Pre-IT	15	0	150	958
	Post-IT	0	0	<15	ND[b]
PFA 36 (TID)	Pre-IT	13	0	100	172
	Post-IT	0	0	<15	<15
PFA 38 (TID)	Pre-IT	364	400	19,952	10,471
	Post-IT	4	1	15	141
PFA 39 (BID)	Pre-IT	110	28	35,481	10,000
	Post-IT	34	0	1,200	240
PFA 50 (BID)	Pre-IT	0	0	20	251
	Post-IT	0	0	<15	158
PFA 56 (TID)	Pre-IT	71	39	1,122	891
	Post-IT	0	0	20	25
PFA 57 (TID)	Pre-IT	900	80	17,943	≥50,000
	Post-IT	0	0	35	316
PFA 59 (BID)	Pre-IT	3	0	446	836
	Post-IT	0	0	<15	<15
PFA 67 (BID)	Pre-IT	13	6	12,511	531
	Post-IT	0	0	<15	<15
PFA 70 (TID)	Pre-IT	115	40	18,721	707
	Post-IT	0	0	<15	1,025
PFA 78 (BID)	Pre-IT	2	0	15	15,848
	Post-IT	0	0	<15	ND
PFA 81 (TID)	Pre-IT	130	12	11,584	39,810
	Post-IT	0	0	20	ND
PFA 86 (BID)	Pre-IT	46	3	1,000	1,025
	Post-IT	0	0	<15	316
PFA 89 (BID)	Pre-IT	680	18	19,952	4,406
	Post-IT	0	0	<15	<15

[a] See footnotes *a* to *c* for Table 1. Ag, antigenemia.
[b] ND, not done (sample not available).

DNA in the eye after IT showed viral DNA clearance in the blood (Table 3).

Antiviral susceptibility assay. Chemosensitivity assays performed on 35 HCMV isolates recovered prior to IT identified no foscarnet-resistant strain, while mean 50, 90, and 99% foscarnet inhibitory doses ± standard deviations were 69.95 ± 28.22, 143.24 ± 38.01, and 216.27 ± 59.20 μM, respectively. The virus isolate from PFA 19, showing stable high levels of antigenemia after IT, was tested for chemosensitivity and found to be in the sensitivity range of all the strains recovered prior to IT. In addition, the patient did not show clinical progression of retinitis.

Clinical efficacy and adverse effects. The effect of foscarnet IT on the clinical and ophthalmoscopic status of retinitis was assessed as no progression for 100% of the patients at the end of IT. Five patients had some progression of retinitis during the first and/or second week of IT. For four of these patients (PFA 20, 56, 78, and 81), aqueous humor samples were available before IT and HCMV DNA levels were very high for three of them (PFA 20, 78, and 81). All of these patients eventually stabilized and continued on maintenance therapy with foscarnet. Reported drug-related adverse events were electrolyte variations (mainly hypocalcemia, hypokalemia, and hypomagnesemia) and two cases of penile ulcerations (one mild and one severe). Electrolyte variations disappeared after pharmacological correction, and the penile ulcerations healed at the end of IT. Of four patients with

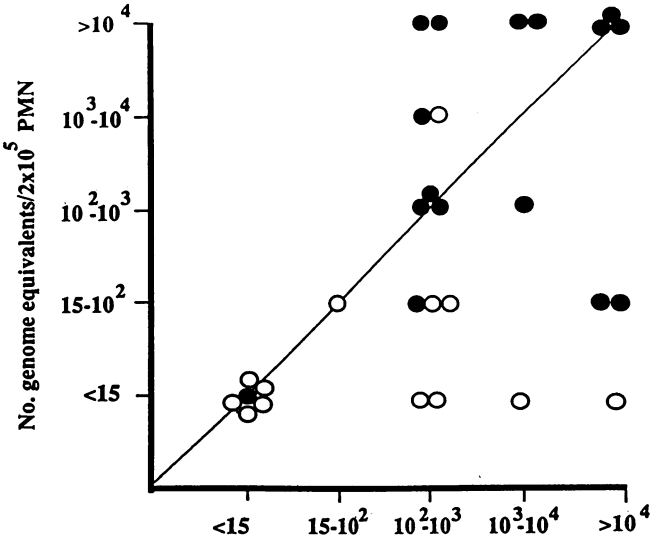

FIG. 2. Correlation of HCMV GE in blood PMNL (PMN) and aqueous humor samples from 16 AIDS patients prior to (●) and from 13 of these same patients after (○) foscarnet IT.

renal failure, three proportionally reduced the drug dosage without discontinuing treatment, whereas one had to stop treatment after 18 days.

DISCUSSION

This investigation showed that of the 65 AIDS patients enrolled in the study only 9 were negative for all HCMV parameters in blood (antigenemia, viremia, and leukoDNAemia). Of these nine patients, six did not suffer from HCMV retinitis (according to retrospective blind examination of ophthalmoscopic pictures taken at the time of enrollment), whereas three patients were shifted from ganciclovir to foscarnet treatment because of ganciclovir myelotoxicity. Thus, it was shown that the blood of all AIDS patients with HCMV retinitis had one or more markers of HCMV infection. Specifically, about half of the patients (30 of 56 [54%]) were positive for all three HCMV markers in their blood, one-fourth (14 of 56 [25%]) for two (antigenemia and leukoDNAemia), and about one-fourth (12 of 56 [21.4%]) for one marker only (leukoDNAemia).

For a precise evaluation of antiviral treatment efficacy, we developed a new PCR method for quantification of HCMV DNA in blood leukocytes. This method is different from the quantification method recently developed in our laboratory and based on the use of internal standards (competitive PCR) (22) and allows HCMV DNA quantification of a large series of clinical samples. Although it uses the same two recombinant DNA molecules previously constructed in the laboratory and used for competitive PCR, the new method is based on the use of external standards in which known increasing amounts of the HCMV IE1 DNA fragment (pCM) are amplified in the presence of a DNA amount equivalent to and in parallel with test samples. Densitometric analysis of hybridization products obtained from the external standards led to construction of a standard curve, from which the amount of DNA in clinical samples was interpolated. In addition, an internal control of amplification consisting of a recombinant DNA molecule amplified by the same set of primers used for HCMV amplification and with the same efficiency of amplification as the HCMV IE1 region was introduced in the PCR assay (22). A small number of copies (n = 100) of this molecule were coamplified with HCMV DNA in both the external standards and clinical samples to detect the presence of PCR inhibitors. While lack of pAC amplification revealed that the results of the PCR assay were not reliable, different levels of pAC amplification products led to normalization of HCMV DNA results during the densitometric analysis of hybridization products.

Such a quantitative DNA method appears highly sensitive for determining very small amounts of viral DNA and thus highly suitable for evaluating the effectiveness of antiviral treatment. However, in order to establish the efficacy of antiviral treatment on HCMV retinitis, quantitation of HCMV in blood leukocytes did not appear to be a reliable parameter. On the other hand, our results suggest that, within the limits of the small numbers examined, all AIDS patients with HCMV retinitis carry viral DNA in the aqueous humor (5) and that such a clinical sample could be used to evaluate the efficacy of antiviral treatment on HCMV retinitis.

The application of our quantitation method to evaluate the efficacy of antiviral IT with foscarnet may have therapeutic implications. Firstly, a 21-day IT with foscarnet was insufficient to clear virus from the blood of all patients treated, since about one-fourth of the patients still had DNA-positive blood leukocytes at the end of IT. This finding, which has not been documented in previous clinical trials, suggests that more prolonged IT should be administered. However, a study investigating whether patients with persisting viral DNA in blood leukocytes are more likely to relapse or progress once maintenance therapy is initiated should be performed before making therapeutic recommendations.

Following IT, of the 12 patients positive in both blood and aqueous humor before IT, 8 became negative for DNA in blood, whereas only 4 became DNA negative for the eye. This finding suggests poor penetration of foscarnet in the eye, as has been already shown for cerebrospinal fluid (18). To obviate this problem, due to systemic limitations of foscarnet dosing, one could argue for prolongation of IT or intravitreal administration of foscarnet (3). However, whether HCMV DNA clearance from the eye is critical may only be clarified by a study attempting to determine whether failure of virologic response may predict earlier progression of retinitis.

Finally, quantitation of viral DNA seems to be useful for careful monitoring of HCMV infections and for deciding antiviral treatment strategies in AIDS patients. This is in contrast to our previous conclusions about immunosuppressed transplant patients (9), for whom quantitations of viremia and antigenemia represent the optimal parameters for a correct monitoring of HCMV infections, whereas quantitation of viral DNA in the late stages of the HCMV infection does not have any significant clinical value (20).

ACKNOWLEDGMENTS

This work was partially supported by Ministero della Sanità, ISS, Progetto Nazionale AIDS 1993, contratto 8205-09, and by Consiglio Nazionale delle Ricerche, PFBTBS, contratto 92.01201.PF70.

We thank Linda D'Arrigo for revision of the English.

REFERENCES

1. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28**:495–503.
2. **Buhles, W. C., Jr., B. J. Mastre, A. J. Tinker, V. Strand, S. H. Koretz, and the Syntex Collaborative Ganciclovir Treatment Study Group.** 1988. Ganciclovir treatment of life- or sight-threatening cytomegalovirus infection: experience in 314 immunocompromised patients. Rev. Infect. Dis. **10**(Suppl. 3):S495–S506.
3. **Diaz-Llopis, M., E. Chipont, S. Sanchez, E. España, A. Navea, and J. L. Menezo.** 1992. Intravitreal foscarnet for cytomegalovirus retinitis in a patient with acquired immunodeficiency syndrome. Am. J. Ophthalmol. **114**:742–747.
4. **Fanning, M. M., S. E. Read, M. Benson, S. Vas, A. Rachlis, V. Kozousek, C. Mortimer, P. Harvey, C. Schwartz, E. Chew, J. Brunton, A. Matlow, I. Salit, H. Vellend, and S. Walmsley.** 1990. Foscarnet therapy of cytomegalovirus retinitis in AIDS. J. Acquired Immune Defic. Syndr. **3**:472–479.
5. **Fenner, T. E., J. Garweg, F. T. Hufert, M. Boehnke, and H. Schmitz.** 1991. Diagnosis of human cytomegalovirus-induced retinitis in human immunodeficiency virus type 1-infected subjects by using the polymerase chain reaction. J. Clin. Microbiol. **29**:2621–2622.
6. **Gerna, G., F. Baldanti, M. Zavattoni, A. Sarasini, E. Percivalle, and M. G. Revello.** 1992. Monitoring of ganciclovir sensitivity of multiple human cytomegalovirus strains coinfecting blood of an AIDS patient by an immediate-early antigen plaque assay. Antiviral Res. **19**:333–345.
7. **Gerna, G., M. G. Revello, E. Percivalle, and F. Morini.** 1992. Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenemia. J.

Clin. Microbiol. **30**:1232–1237.

8. **Gerna, G., M. G. Revello, E. Percivalle, M. Zavattoni, M. Parea, and M. Battaglia.** 1990. Quantification of human cytomegalovirus viremia by using monoclonal antibodies to different viral proteins. J. Clin. Microbiol. **28**:2681–2688.
9. **Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zavattoni, P. Grossi, and G. Milanesi.** 1991. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia and DNAemia. J. Infect. Dis. **164**:488–498.
10. **Holland, G. N., Y. Sidikaro, A. E. Kreiger, D. Hardy, M. J. Sakamoto, L. M. Frenkel, D. J. Winston, M. S. Gottlieb, Y. J. Bryson, R. E. Champlin, W. G. Ho, R. E. Winters, P. R. Wolfe, and J. D. Cherry.** 1987. Treatment of cytomegalovirus retinopathy with ganciclovir. Ophthalmology **94**:815–823.
11. **Jabs, D. A., C. Newman, S. De Bustros, and B. F. Polk.** 1987. Treatment of cytomegalovirus retinitis with ganciclovir. Ophthalmology **94**:824–830.
12. **Jacobson, M. A., J. J. O'Donnell, and J. Mills.** 1989. Foscarnet treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. Antimicrob. Agents Chemother. **33**:736–741.
13. **Jacobson, M. A., J. J. O'Donnell, D. Porteous, H. R. Brodie, D. Feigal, and J. Mills.** 1988. Retinal and gastrointestinal disease due to cytomegalovirus in patients with the acquired immune deficiency syndrome: prevalence, natural history, and response to ganciclovir therapy. Q. J. Med. **67**:473–486.
14. **Laskin, O. L., D. M. Cederberg, J. Mills, L. J. Eron, D. Mildvan, and S. A. Spector.** 1987. Ganciclovir for the treatment and suppression of serious infections caused by cytomegalovirus. Am. J. Med. **83**:201–207.
15. **Lehoang, P., B. Girard, M. Robinet, P. Marcel, L. Zazoun, S. Matheron, W. Rozenbaum, C. Katlama, I. Morer, J. O. Lernestedt, H. Saraux, Y. Pouliquen, M. Gentilini, and F. Rousselie.** 1989. Foscarnet in the treatment of cytomegalovirus retinitis in acquired immune deficiency syndrome. Ophthalmology **96**:865–874.
16. **Palestine, A. G., M. A. Polis, M. D. De Smet, B. F. Baird, J. Falloon, J. A. Kovacs, R. T. Davey, J. J. Zurlo, K. M. Zunich, M. Davis, L. Hubbard, R. Brothers, F. L. Ferris, E. Chew, J. L. Davis, B. I. Rubin, S. D. Mellow, J. A. Metcalf, J. Manischewitz, J. R. Minor, R. B. Nussenblatt, H. Masur, and H. C. Lane.** 1991. A randomized, controlled trial of foscarnet in the treatment of cytomegalovirus retinitis in patients with AIDS. Ann. Intern. Med. **115**:665–673.
17. **Palestine, A. G., G. Stevens, Jr., H. C. Lane, H. Masur, L. S. Fujikawa, R. B. Nussenblatt, A. H. Rook, J. Menischewitz, B. Baird, M. Megill, G. Quinnan, E. Gelmann, and A. S. Fauci.** 1986. Treatment of cytomegalovirus retinitis with dihydroxypropoxymethyl guanine. Am. J. Ophthalmol. **101**:95–101.
18. **Sjövall, J., S. Bergdahl, G. Movin, S. Ogenstad, and M. Saarimäki.** 1989. Pharmacokinetics of foscarnet and distribution to cerebrospinal fluid after intravenous infusion in patients with human immunodeficiency virus infection. Antimicrob. Agents Chemother. **33**:1023–1031.
19. **Studies of Ocular Complications of AIDS Research Group, in collaboration with the AIDS Clinical Trials Group.** 1992. Mortality in patients with the acquired immunodeficiency syndrome treated with either foscarnet or ganciclovir for cytomegalovirus retinitis. N. Engl. J. Med. **326**:213–220.
20. **van den Berg, A. P., W. J. van Son, R. A. J. Janssen, N. H. C. Brons, A. A. Heyn, A. Scholten-Sampson, S. Postma, M. van der Giessen, A. M. Tegzess, L. H. F. M. de Leij, and T. H. The.** 1992. Recovery from cytomegalovirus infection is associated with activation of peripheral blood lymphocytes. J. Infect. Dis. **166**:1228–1235.
21. **Walmsley, S. L., E. Chew, S. E. Read, H. Vellend, I. Salit, A. Rachlis, and M. M. Fanning.** 1988. Treatment of cytomegalovirus retinitis with trisodium phosphonoformate hexahydrate (foscarnet). J. Infect. Dis. **157**:569–572.
22. **Zipeto, D., F. Baldanti, D. Zella, M. Furione, A. Cavicchini, G. Milanesi, and G. Gerna.** 1993. Quantification of human cytomegalovirus DNA in peripheral blood leukocytes of immunocompromised patients by the polymerase chain reaction. J. Virol. Methods **44**:45–56.