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Quantitative systemic and local evaluation of the antiviral effect of ganciclovir and foscarnet induction treatment on human cytomegalovirus gastrointestinal disease of patients with AIDS

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Abstract

In a group of 29 AIDS patients with biopsy-proven human cytomegalovirus (HCMV) gastrointestinal disease (GID), HCMV GID was shown to correlate mostly with systemic HCMV infection. The antiviral induction treatment (IT) with either ganciclovir (GCV) or foscarnet (PFA) caused a significant reduction in the level of HCMV antigenemia, viremia and leukoDNAemia, and a complete virus clearance or a sharp drop of viral load in the blood of 13/13 patients and in the gastrointestinal (GI) mucosa of 12/13 (92%) patients in the GCV arm, and in the blood of 13/14 (93%) patients and in the GI mucosa of 10/12 (83%) patients in the PFA arm of the study, respectively. Similarly, the clinical response was good in 13/15 (87%) patients in the GCV arm and in 13/14 (93%) patients in the PFA arm. In addition, the finding that 2/6 patients positive for HCMV isolated from both GI mucosa and blood prior to IT were still positive in the GI tract after IT, suggested that IT could be prolonged to clear the virus from GI tract. In conclusion, both GCV and PFA showed a remarkable systemic and local antiviral effect in the treatment of HCMV GID in AIDS patients. © 1997 Elsevier Science B.V.

Keywords: AIDS; Human cytomegalovirus; Gastrointestinal disease; Ganciclovir; Foscarnet; Antivirals

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[1] This work was carried out in association with the members of the Italian Foscarnet GID Study Group (a full list of centers and contributing investigators is included in Appendix A)

1. Introduction

Human cytomegalovirus (HCMV) disseminated disease is found at autopsy in up to 90% of

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patients with AIDS (Drew, 1992) representing a major cause of morbidity and mortality. Although retinitis is the most common organ localization of HCMV disease, gastrointestinal disease (GID) of both the upper and lower tract is reported as occurring with a frequency of $\leq 15\%$ (Jacobson and Mills, 1988). Diagnosis of HCMV GID is often difficult (Blanshard et al., 1995) and multiple criteria, i.e., clinical, endoscopic, histologic and virologic, must be adopted for a correct diagnosis (Cotte et al., 1993). Early diagnosis is mandatory to initiate a timely antiviral treatment.

The present study evaluates the following issues in a group of 29 AIDS patients with biopsy-proven HCMV GID: (i) the correlation between HCMV GID (lower or upper) and levels of HCMV viremia, antigenemia and leukoDNAemia; (ii) the effect of antiviral induction treatment (IT) with either ganciclovir (GCV) or foscarnet (PFA) on virologic and clinical responses; (iii) the potential anti-HIV effect of PFA. Results indicate that: (i) in most patients HCMV disease correlates locally with systemic HCMV infection; (ii) IT with either GCV or PFA is highly effective both at a systemic and local level; (iii) the anti-HIV effect of PFA IT was not shown to be significant in the restricted number of patients studied.

2. Materials and methods

2.1. Patients

Twenty-nine patients with AIDS were enrolled in the study according to the following inclusion criteria: endoscopically- and histologically-proven first episode of HCMV upper (esophagitis, gastritis) or lower (colitis, proctitis) GID; documented HIV infection/disease; >18 years of age; exclusion of current or potential pregnancy; and informed consent. Exclusion criteria were: presence of other concomitant gastrointestinal (GI) infection (fecal samples for bacterial cultures, and for ova and parasites, were obtained and assessed before inclusion of patients in the study); creatinine clearance <60 ml/min; absolute neutrophil count $<750/\mu$l; hemoglobin <80g/l; platelets $<25\,000/\mu$l; treatment with potentially nephrotoxic drugs (aminoglycosides, amphotericin B, intravenous pentamidine, intravenous cotrimoxazole); concomitant treatment with AZT, DDI, DDC, GMCSF, GCSF, interferon or any other experimental drug; presence of severe respiratory or neurologic disease; <3 months quoad vitam prognosis apart from HCMV GID; Karnovsky <50; treatment with HCMV drugs in the last months before inclusion; known hypersensitivity to GCV or PFA; and breast-feeding. All patients were enrolled in 10 different Infectious Disease Centers (in association with local gastroenterology and pathology centers) in Northern Italy and were stratified into 2 groups: upper GID (esophagitis, gastritis, duodenitis) or lower GID (colitis, proctitis). The protocol was approved by the local ethics committees and all subjects gave written informed consent.

2.2. Antiviral treatment

Patients enrolled in each group were randomized to receive either GCV (5 mg/kg twice daily) or PFA (90 mg/kg twice daily) for a 3-week period. GCV was administered as a 1 h infusion, whereas PFA as a 2 h infusion with 750–1000 ml of normal saline to reduce the risk of renal damage (Deray et al., 1989). Treatment with antiretroviral drugs was discontinued during the study.

2.3. Quantitation of clinical and laboratory parameters

Before initiation and at the end of IT clinical and laboratory parameters were evaluated and quantified. Clinical symptoms associated with HCMV GID of the upper (nausea, vomiting, restrosternal pain, food sticking, pain at swallowing) or lower (nausea, vomiting, abdominal pain) GI tract were each quantified according to the following scores: 0, absent; 1, mild; 2, moderate; 3, severe. The number of daily stools, their consistency and the presence or absence of blood was recorded. Macroscopic lesions were evaluated during endoscopic examination and quantified. For the upper GI tract, 0 indicated normal mucosa; 1, erythema/friability; 2, <3 separate ero-

sions; 3, > 3 separate erosions or confluent circular erosions; 4, ulcers present. For the lower GI tract: 0, indicated normal mucosa; 1, distorted or absent mucosal vascular pattern/granularity; 2, erythema/friability; 3, single or multiple small (< 5 mm) ulcers, fewer than 10 per 10 cm segment; 4, large ulcer (> 5 mm) or more than 10 ulcers per 10 cm fragment. Prior to IT, at least three mucosa biopsy samples were routinely taken at the level of each mucosal lesion and, sometimes, of normal mucosa. At the end of IT, mucosal biopsies were taken from any area of abnormality and from the areas where HCMV had been found prior to IT. Histopathology was quantified on biopsy samples by counting the number of intranuclear inclusion-bearing cells (IIBC) per section (at least two levels per block were examined) and by evaluating the degree of inflammation. In detail, IIBC per section were scored as follows: 0, absent; 1, < 5; 2, 5–10; 3, > 10; 4, presence of IIBC in an ulcer. The degree of inflammation was scored as follows: 0, absent; 1, mild; 2, moderate; 3, marked; 4, presence of ulcers.

2.4. Quantitation of virologic parameters

HCMV quantitation in blood was achieved on peripheral blood leukocytes (PBL) by performing the following assays, which have been previously reported and shown to be highly sensitive for HCMV quantitation and evaluation of antiviral treatment effectiveness (Gerna et al., 1994). The viremia assay was performed by inoculating 2×10^5 PBL onto a monolayer of human embryonic lung fibroblasts in a shell vial. Following 24 h incubation, cells were fixed and stained by immunofluorescence using a monoclonal antibody reactive with the 72 kD major immediate-early protein. The number of infected cells showing stained nuclei was counted and recorded as the number of inoculated PBL carrying infectious virus (Gerna et al., 1990). The antigenemia assay was performed using cytospin preparations of 2×10^5 PBL which were stained with a pool of 3 monoclonal antibodies reactive with 3 different epitopes of the HCMV lower matrix phosphoprotein pp65. Quantitation of antigenemia was achieved by counting the number of pp65-positive nuclei/2×10^5 PBL examined (Gerna et al., 1992). Quantitation of HCMV DNA in PBL or leukoDNAemia (L-DNA) was achieved by a PCR method previously described (Gerna et al., 1994). The amount of viral DNA was expressed as the number of HCMV genome equivalents (GE)/1×10^5 PBL examined. Conventional HCMV isolation was routinely performed on blood using aliquots of 2×10^5 PBL and, whenever possible, on the GI tract using GI biopsy samples.

In addition, HIV-1 genomic RNA was quantified in plasma from 9 patients treated with PFA before and at the end of IT. In parallel, 8 additional patients treated with GCV were tested for quantitative HIV-1 genomic RNA determination. Quantitation of HIV-1 plasma viremia was achieved by reverse transcription-PCR using the previously reported methodology by Menzo et al. (1992) and reagents kindly provided by the same authors. Furthermore, all 17 patients were assayed for quantitation of serum HIV-1 p24 antigen following immune complex dissociation by using a commercial enzyme-linked immunosorbent assay (Coulter, Hiealeah, FL).

2.5. Evaluation of efficacy

The evaluation of the virologic and clinical response to antiviral treatment was performed as reported below. The virologic response in blood was considered complete or good when HCMV clearance or > 90% reduction in viral load was evidenced by all assays. The local response to antiviral treatment in the GI mucosa was evaluated as follows: 1, complete response, corresponding to disappearance of IIBC; 2, good response, when the number of IIBC decreased by at least 2 steps; 3, partial response, when the decrease in number of IIBC was limited to one step; 4, no change in number of IIBC; 5, increase in number of IIBC. The evaluation of the clinical response adopted the same scoring system with respect to baseline GI symptoms: 1, disappearance (complete response); 2, > 50% improvement (good response); 3, < 50% improvement (partial response); 4, no change; 5, progression. In addition, the macroscopic (endoscopic) and the microscopic response (with respect to inflammation) were eval-

Table 1
Pre-IT clinical and virologic findings in 15 AIDS patients with biopsy-proven GID treated with ganciclovir

Pt. no., GID site, months after AIDS diagnosis ($CD_4/\mu l$)	HCMV quantitation in blood			GI biopsy lesion score[a]		
	Ag.	Vir.	L-DNA (no. GE)	Macr.	Micr.	
					Incl.	Infl.
G 2, U, 12 (5)	93	10	2558	4	1	2
G 15, L, 0 (12)	9	3	551	4	2	4
G 18, U, 5 (111)	0	0	35	4	1	3
G 25, U, 0 (36)	0	0	<15	4	1	2
G 33, U, 15 (6)	157	ND	3398	3	1	4
G 35, L, 1 (17)	40	ND	316	1	1	2
G41, U, 0 (87)	12	0	175	4	2	2
G 47, U, 0 (120)	29	4	400	3	1	4
G 50, U, 24 (5)	68	15	15 850	1	2	2
G 54, U, 1 (17)	16	0	3981	2	1	2
G 56, U, 15 (7)	18	5	2100	4	2	3
G 57, L, 0 (30)	11	2	2118	1	3	3
G 58, L, 9 (3)	1000	185	40 000	4	2	2
G 61, U, 0 (44)	0	0	<15	4	2	4
G 118, U+L, 5 (12)	7	0	631	2	3	2

Abbreviations: Ag., antigenemia; Vir., viremia; L-DNA, leukoDNAemia; GE, genome equivalents; Macr., macroscopic (endoscopic) evaluation of GI lesions; Micr., microscopic evaluation of GI biopsy; Incl., inclusion bodies.; Infl., inflammation; ND, not done; U, upper GID; L, lower GID; IT, induction treatment.
[a] Score 0–4, from no lesion to most severe lesions, as reported in Section 2.

uated according to the same criteria. Thus, a complete or good response was obtained with scores of 1 or 2, a partial or absent response with scores of 3 or 4, respectively.

2.6. Statistical analysis.

Means of paired data were compared by using the Wilcoxon matched pair test, whereas means of unpaired data were compared by using the Kolmogorow-Smirnov two sample test. Difference in distribution was evaluated by using the Fisher's exact test.

3. Results

3.1. Pre-IT correlation of HCMV presence in GI mucosa and blood

IIBC presence was the major criterion for defining the pathogenic role of HCMV in GI mucosa and for selecting patients. On the whole, 29 patients completed the study: 15 in the GCV and 14 in the PFA arm. In the GCV group, GID was relevant to the lower GI tract in 4 patients, whereas it involved the upper tract in 10 patients and both tracts in the last patient. The time elapsed after AIDS diagnosis was 0–24 months with a median of 1 month. The IIBC score was in the range of 1–3. Virus was detected in blood by viremia, antigenemia, and L-DNA in 7 patients, by antigenemia and L-DNA in 5 patients, by L-DNA only in one patient, whereas it was not detected at all in 2 patients (Table 1).

On the other hand, in the PFA group GID involved the upper GI tract in 12 patients, the lower tract in one patient and both tracts in an additional patient. Time elapsed after AIDS diagnosis was 0–12 months with a median of 3 months. Again, the IIBC score was in the range of 1–3. HCMV was detected in blood by all 3 assays in 11 patients and by 2 assays (antigenemia and DNAemia) in the remaining 3 patients (Table 2).

Table 2
Pre-IT clinical and virologic findings in 15 AIDS patients with biopsy-proven GID treated with foscarnet

Pt. no., GID site, months after AIDS diagnosis ($CD_4/\mu l$)	HCMV quantitation in blood			GI biopsy lesion score[a]		
	Ag.	Vir.	L-DNA (no. GE)	Macr.	Micr.	
					Incl.	Infl.
G 1, U, 5 (8)	650	36	17 080	1	3	4
G 11, U, 7 (6)	210	48	10 000	4	3	3
G 19, U, 2 (16)	4	0	105	4	1	4
G 24, U, 8 (1)	94	31	19 992	4	1	4
G 37, U+L, 0 (4)	29	3	1588	2	3	4
G 38, U, 0 (22)	6	3	281	4	2	4
G 40, U, 3 (77)	1	0	80	4	2	4
G 52, U, 12 (19)	800	10	17 783	1	3	2
G 107, U, 0 (14)	18	1	3270	4	3	2
G 114, U, 0 (24)	15	9	720	4	3	4
G 126, U, 3 (6)	118	40	3820	2	3	3
G 128, U, 2 (23)	9	0	540	4	3	3
G 204, L, 4 (13)	123	43	4870	4	2	4
G 301, U, 12 (5)	11	11	40	4	3	4

[a] See Table 1.

Prior to IT, GI mucosa biopsy samples were available for virus isolation from 11 patients with IIBC who completed the study as well as from 4 patients (G10, G14, G34, G62) positive for IIBC who could not be enrolled in the study (Table 3). Using GI samples, HCMV was isolated from 7 patients (47%) and HSV from 3 patients, whereas no virus was recovered from 5 patients. In a single patient (G47 of the GCV arm) both HCMV and HSV were isolated: HCMV according to the rapid shell vial assay and HSV according to the conventional isolation procedure. In all but 2 of these 15 patients HCMV was recovered from blood, whereas it was isolated from the biopsy sample of one of the 2 patients negative for virus isolation from blood. Thus, on the whole, HCMV was recovered from the GI tract of 6/13 patients with IIBC and infectious virus in blood, and of 1/2 patients with IIBC and no infectious virus in blood (Table 4).

In addition, 22 patients with AIDS and GI symptoms underwent GI biopsy for potential inclusion in the study. All of them were negative for IIBC and, thus, excluded from the study. However, it appeared interesting to examine virus isolation findings obtained on GI biopsy samples and blood from these patients. In fact, HCMV was recovered from GI biopsy samples negative for IIBC of 3/5 patients with positive isolation from blood (adenovirus was recovered from the other 2 patients), but it was also isolated from 2/17 patients negative for either IIBC in GI mucosa samples or HCMV isolation from blood (Table 4). On the whole, no significant difference in virus isolation rate was found between patient groups with and without IIBC, whereas the virus isolation rate was significantly higher ($P < 0.05$) in the group of patients with HCMV in blood as compared to the group of patients without HCMV in blood (Table 4).

3.2. Virologic evaluation of the effect of antiviral treatment

The virologic response to antiviral treatment was evaluated as follows: (i) systemically, on blood, by quantifying levels of viremia, antigenemia and L-DNA before and after IT; (ii) locally, on GI biopsy samples by quantifying the number of IIBC per histologic section of biopsy tissue before and after IT. No virologic parameter was considered more suitable than IIBC for a reliable

Table 3
Correlation of HCMV inclusion bearing cells in GI mucosa, viremia and virus isolation from blood, and HCMV isolation from GI biopsy samples in 15 AIDS patients with GID

Pt. no.	Blood sample taken	Presence of infectious HCMV in blood		GID site, inclusions in GI mucosa (no./biopsy)	Virus isolation from GI biopsy samples	
		Viremia (no. pos. cells)[a]	Virus isolation		Rapid (shell vial)	Conventional
G1	Pre-IT	Pos (36)	Pos	Upper, pos (>10)	Neg	HSV
G2	Pre-IT	ND	Pos	Upper, pos (<5)	ND	HSV
G10	Pre-IT	Pos (1)	Neg	Upper, pos (<5)	HCMV	HCMV
	Post-IT	Neg	Neg	Neg	Neg	Neg
G14	Pre-IT	Pos (4)	Pos	Upper, pos (<5)	Neg	Neg
G15	Pre-IT	Pos (3)	Pos	Lower, pos (8)	HCMV	HCMV
	Post-IT	Neg	Neg	Neg	Neg	Neg
G24	Pre-IT	Pos (31)	Pos	Upper, pos (<5)	HCMV (u+n)	HCMV (u+n)
	Post-IT	Neg	Neg	ND	HCMV (u)	HCMV (u)
G25	Pre-IT	Neg	Neg	Upper, pos (<5)	Neg	Neg
	Post-IT	Neg	Neg	Neg	Neg	Neg
G34	Pre-IT	Pos (10)	Pos	Upper, pos (>10)	HSV	HSV
G35	Pre-IT	Pos (3)	Pos	Lower pos (<5)	Neg	Neg
	Post-IT	Neg	Neg	Neg	Neg	Neg
G40	Pre-IT	Neg	Neg	Upper, pos (7)	HCMV	Neg
	Post-IT	Neg	Neg	Neg	Neg	Neg
G41	Pre-IT	Neg	Pos	Upper, pos (9)	HCMV (u+n)	HCMV (u+n)
	Post-IT	Neg	Neg	ND	HCMV (u)	HCMV (u)
G47	Pre-IT	Pos (4)	Neg	Upper, pos (<5)	HCMV	HSV
	Post-IT	Neg	Neg	Neg	Neg	Neg
G54	Pre-IT	Neg	Pos	Upper, pos (<5)	Neg	Neg
	Post-IT	Neg	Neg	Neg	Neg	Neg
G56	Pre-IT	Pos (5)	Pos	Upper, pos (7)	Neg	Neg
	Post-IT	Neg	Neg	Neg	Neg	Neg
G62	Pre-IT	Pos(3)	Pos	Lower, pos (>10)	HCMV	HCMV

Abbreviations: IT, induction treatment; u, ulcer; n, normal mucosa; HSV, herpes simplex virus.
[a] No. p72-positive fibroblast nuclei/2×10^5 PBL inoculated into a shell vial.

Table 4
HCMV isolation rate from GI biopsy samples taken from AIDS patients with suspected GID

Histology	HCMV isolation (%)			P^a
	HCMV in blood	Lack of HCMV in blood	Total	
w IIBC[b]	6/13 (46)	1/2 (50)	7/15 (47)	NS
w/o IIBC	3/5 (60)	2/17 (12)	5/22 (23)	0.055
Total	9/18 (50)	3/19 (16)	12/37 (32)	<0.05
P^a	NS	NS	NS	

[a] Fisher's exact test. NS, not significant.
[b] IIBC, intranuclear inclusion-bearing cells.

evaluation of the local effect. At the end of IT a highly significant decrease in levels of viremia, antigenemia and L-DNA was observed in both GCV and PFA arms of the study (Table 5). On the other hand, no significant difference was found in pre-IT as well as post-IT levels of different viral parameters between the two arms of the study (Table 5).

In detail, in the GCV arm 12/12 patients for antigenemia, 7/7 patients for viremia and 13/13 patients for DNAemia showed a complete or good response. Thus, on the whole, all patients (13/13) responded virologically to GCV IT (Table 6). In the PFA arm 13/14 patients for antigenemia, 10/11 patients for viremia and 12/14 patients for DNAemia gave a complete or good response (Table 6). Thus, PFA IT was highly effective in eliminating or sharply decreasing HCMV or HCMV markers in blood of 13/14 patients (93%). In patient G52 (Table 2) levels of all 3 viral parameters remained unaltered at the end of IT, even though the HCMV isolates recovered before and after IT were susceptible to PFA.

On the other hand, the local response at the GI level was found to be complete or good (disappearance or 2 grade reduction in number of IIBC) in 12/13 (92%) patients in the GCV arm, and in 10/12 (83%) patients in the PFA arm (Table 6). As for virus recovery, HCMV was isolated from GI biopsy samples in 6/10 (60%) patients tested before IT (5 blood-positive and one blood-negative for HCMV isolation), whereas HCMV was not recovered in the remaining 4 patients (3 blood-positive and one blood-negative) (Table 3). Following IT, of the 6 patients HCMV-positive in the GI mucosa (3 were treated with GCV and 3 with PFA), 2 (1 treated with GCV and 1 with PFA) were still HCMV-positive in the GI tract after IT, even though to a lesser extent. However, in the meantime, both patients became negative for virus recovery from blood, as also noted for the other 4 patients clearing virus from GI tract (Table 3).

3.3. Clinical, macroscopic and microscopic response

The clinical response was complete or good in 13/15 patients (87%) in the GCV arm, and in 13/14 (93%) patients in the PFA arm (Table 6). Macroscopic (endoscopic) and microscopic (inflammation process) responses were less significant: 73% (11/15 patients) and 54% (7/13) in the GCV, and 64% (9/14) and 50% (6/12) in the PFA arm, respectively (Table 6). No significant difference in the percentage of responders was found between the two arms of the study for any of the virologic and clinical parameters considered (Table 6).

3.4. Evaluation of the anti-HIV-1 effect of PFA IT

The potential anti-HIV-1 effect of PFA was evaluated in 9 patients with AIDS and HCMV GI localization who underwent PFA IT by comparing levels of plasma HIV-1 RNA and p24 antigen before and after IT (Table 7). A control group consisted of 8 additional patients with AIDS and

Table 5
Virologic response to GCV and PFA IT in blood of 15 and 14 patients with AIDS and biopsy-proven GID respectively

HCMV virologic parameter	Blood sample taken	GCV median value (range)	PFA median value (range)	P^a
Antigenemia	Pre-IT	16 (0–1000)	23.5 (0–800)	NS
	Post-IT	0 (0–7)	0 (0–1000)	NS
P^b		0.002	0.01	
Viremia	Pre-IT	2 (0–185)	9.5 (0–48)	NS
	Post-IT	0	0	NS
P^b		0.018	0.016	
DNAemia	Pre-IT	631 (<15–40 000)	2429 (40–19 992)	NS
	Post-IT	<15 (<15–40)	<15 (<15–11 220)	NS
P^b		0.001	0.001	

[a] Kolmogorow-Smirnov two sample test. NS, not significant.
[b] Wilcoxon matched pair test.

HCMV GI localization who were studied for the same HIV-1 parameters prior to and after GCV IT. A slight decrease in median levels of plasma HIV-1 RNA and p24 antigen was observed in patients treated with PFA IT. Mean HIV RNA plasma viremia as well as HIV p24 antigen levels slightly decreased in the PFA IT (4.12 versus 4.03 $\log_{10}$ HIV RNA copies, and 213.7 versus 157.6 pg/ml, respectively) compared to the GCV IT group, in which the relevant levels slightly increased during treatment (3.36 versus 3.79 $\log_{10}$ HIV RNA copies and 180.7 versus 201.0 pg/ml, respectively). However, no statistically significant difference between pre-IT and post-IT mean levels of both parameters was observed in either group of patients (Table 7).

Table 6
Response to GCV or PFA IT in AIDS patients with GID

HCMV	Responders[a]/total patient number (%) after IT with		P^b
	GCV	PFA	
Viral load[c]	13/13 (100)	13/14 (93)	NS
Antigenemia[d]	12/12 (100)	13/14 (93)	NS
Viremia[d]	7/7 (100)	10/11 (91)	NS
DNAemia[d]	13/13 (100)	12/14 (86)	NS
Endoscopy[e]	11/15 (73)	9/14 (64)	NS
Microscopy			
IIBC[f]	12/13 (92)	10/12 (83)	NS
Inflammation[e]	7/13 (54)	6/12 (50)	NS
Clinical[e]	13/15 (87)	13/14 (93)	NS

[a] Patients showing a complete or good response according to criteria reported in Section 2.
[b] Fisher's exact test. NS, not significant.
[c] ≥90% reduction in level as determined by all 3 assays.
[d] ≤90% reduction in level.
[e] Disappearance or >50% improvement of endoscopic lesions, inflammation or clinical symptoms, respectively.
[f] IIBC, intranuclear inclusion-bearing cells. Disappearance or reduction in IIBC number from >10 to <5/section.

4. Discussion

Three major issues were addressed in this study: (i) the diagnosis of HCMV GID and its correlation with systemic HCMV infection (presence of HCMV in blood); (ii) the evaluation of the virologic response to antiviral treatment with either GCV or PFA, both at the local (GI mucosa) and systemic (blood) level; (iii) the contextual evaluation of the potential anti-HIV effect of PFA IT in a minor number of patients who received PFA because of HCMV GID.

Table 7
Effect of GCV or PFA IT on HIV plasma RNA and p24 levels of 9 and 8 AIDS patients with HCMV disseminated infection and GID respectively

Patient group	Blood sample taken	HIV median levels (range)	
		Plasma RNA (log_{10} GE/ml)[a]	p24 (pg/ml)
PFA, 9 patients	Pre-IT	6.17 (4.6–6.39)	112.5 (<20–775)[b]
	Post-IT	5.69 (4.3–6.39)	62.0 (<20–648)[c]
P[d]		NS	NS
GCV, 8 patients	Pre-IT	5.42 (4.3–6.17)	154.0 (<20–517)[b]
	Post-IT	5.19 (4.3–6.3)	78 (<20–634)[c]
P[d]		NS	NS

[a] GE, genome equivalents.
[b] 1 patient was negative (<20 pg/ml).
[c] 2 patients were negative.
[d] Wilcoxon matched pair test.

The first issue regards the well-known difficulty in diagnosing HCMV GID based on the contextual evaluation of clinical, endoscopic, histologic and virologic findings (Cotte et al., 1993). The following conclusions can be drawn from the data reported in this study: (i) infectious HCMV is detected both in GI mucosa and blood of the majority of patients with AIDS and GID, while other DNA viruses, such as HSV or adenovirus, may mask HCMV during the virus isolation procedure from the GI tract; (ii) in a minority of patients with AIDS and GID, infectious HCMV may be present in the GI tract and cause disease without being detectable in blood; (iii) a close correlation does not seem to exist between IIBC in GI mucosa and HCMV isolation from GI biopsy samples.

HCMV GID in patients with AIDS must be considered a local manifestation of a systemic disease. Thus, we believe that it is crucial to evaluate the presence of HCMV in both blood and GI mucosa not only on a qualitative, but also on a quantitative basis. Nonetheless, despite well defined HCMV quantification methods in blood through determination of HCMV antigenemia, viremia (Gerna et al., 1990) and L-DNA (Gerna et al., 1994), HCMV quantification in GI mucosa appears to be more complicated. The major drawback in local HCMV quantitation by either PCR or titration of infectious virus derives from the risk that results are altered by HCMV presence in contaminating blood (leukocytes or plasma). Recently, a paper was published correlating high HCMV DNA levels in GI biopsy samples with HCMV GID in AIDS patients (Cotte et al., 1993). However, viral DNA was not quantified by the same PCR method in blood and the presence of infectious virus in blood of nearly all AIDS patients with GID contributed to strengthen the suspicion that the local HCMV DNA results could be biased by the virus present in blood. In the present study the virus isolation rate in patients with HCMV in blood was significantly higher than in the group of patients without HCMV in blood. For this reason, we preferred to program our study to rely upon IIBC quantitation rather than quantifying virus by PCR in GI samples as we did in blood leukocytes.

It has been shown that IIBC are, by far, less common than an atypical cytopathic effect (Francis et al., 1989) and that histology has a sensitivity of 30–54% compared to HCMV recovery from GI biopsies, while both histology and cultures show a sensitivity of 38–58% compared to in situ

hybridization (Clayton et al., 1989; Roberts et al., 1988; Wu et al., 1989). However, inadequate tissue sampling may partially account for the low sensitivity of routine histology in these studies (Hackman et al., 1994). In our series the comparable sensitivity of IIBC and culture (40 versus 32%) in detecting HCMV in the GI mucosa was partly explained by the relatively poor HCMV-specificity of IIBC which was documented by the comparable ($P > 0.05$) HCMV isolation rate in patients with and without IIBC and, on the other hand, by the recovery of HSV from GI samples of some patients with IIBC. However, the strong local response to antiviral treatment obtained in either the GCV or the PFA arm of the study in terms of disappearance or sharp drop in number of IIBC in the great majority ($\geq 85\%$) of patients indicates that IIBC represent a valuable parameter for diagnosing HCMV GID. The good clinical response obtained in parallel substantiates this conclusion.

On the other hand, the virologic response was striking when considered on blood virologic parameters determined prior to and after IT with either GCV or PFA. These results appear somewhat comparable to those previously reported by our group on the effect of PFA IT on quantitation of HCMV in PBL and aqueous humor of patients with AIDS and HCMV retinitis (Gerna et al., 1994). However, as previously reported in the HCMV retinitis study, the local effect of antiviral IT in the GID study was slower to develop than the systemic effect, at least in a minority of patients, thus suggesting the need for more prolonged treatment to achieve local virus clearance. On the whole, clinical and local results of our study are largely comparable to those obtained by Blanshard et al. (1995) in the first randomized comparative study of GCV and PFA in the IT of HCMV GID. However, this study did not pay attention to the effect of antiviral treatment on virus in blood.

In the GID series of patients, only a single patient did not respond at all to IT virologically, and this was in the PFA arm of the study (patient G52). Antiviral susceptibility testing of HCMV strains present in blood of this patient by the conventional plaque-reduction assay using viral isolates (Sarasini et al., 1995) as well as by the rapid screening for resistance of primary isolates of HCMV from culture-positive blood samples (Gerna et al., 1995) showed an apparent normal susceptibility to PFA. Whether the discrepancy between the in vivo resistance and the in vitro susceptibility of HCMV strains from this patient could be due to the possible presence of a mixed viral population or to the recently reported delay in viral growth of HCMV strains showing PFA-resistance (Baldanti et al., 1996) remains to be determined.

Finally, the repeatedly reported (both in vitro and in vivo) anti-HIV effect of PFA (Sandstrom et al., 1985; Gaub et al., 1987; Bergdahl et al., 1988; Jacobson et al., 1988; Studies of Ocular Complication of AIDS Research Group and the AIDS Clinical Trial Group, 1992; Reddy et al., 1992; Fletcher et al., 1994) was not confirmed in a small number of patients treated with PFA IT compared to an equally small number of patients treated with GCV IT (controls). In fact, the slight reduction (26%) in serum HIV p24 mean level as well as in HIV-1 plasma RNA (19%) observed in the PFA IT group was not found to be statistically significant.

In conclusion, the reported study shows that: (i) HCMV GID is mostly associated with systemic HCMV infection, while IIBC seem to represent a sufficiently reliable parameter for diagnosing HCMV GID; (ii) both GCV and PFA IT are highly effective in inducing a good virologic response in the great majority of patients with AIDS and HCMV GID; and (iii) an anti-HIV effect of PFA IT was not seen for either HIV-1 serum p24 antigen and plasma RNA levels.

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Appendix A

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