

E. Gómez¹, A. S. Laurés¹, S. Melón²,
J. M. Baltar¹, M. de Oña²

Late CMV Disease and/or Recurrence in Renal Transplants Recipients with Prolonged Oral Ganciclovir Prophylaxis

Background/Aim: Oral ganciclovir effectively prevents CMV disease, but late-onset CMV disease is increasingly observed in pancreas-kidney and bone-marrow transplants recipients, specially with long-term prophylaxis. Then, the aim of this study was to verify if prolonged administration of oral ganciclovir prophylaxis could induce late CMV recurrence or disease in renal transplant recipients

Material/Methods: 64 renal transplant recipients with a follow-up of 1 year were divided in two groups: 27 patients of short-term group (ST-group) treated 1-2 months with oral GCV prophylaxis, and 37 patients of long-term group (LT-group) treated 3-4 months. CMV Antigenemia and PCR were performed weekly until 4 first months after transplant, and monthly until the end of the study.

Results: At 4 months, CMV replication was similar in ST-group and LT-group (33% vs. 32.4%, respectively), but at 1 year, was found more frequently in ST-group than in LT-group (77.8% vs 51.4%, respectively, $p=0.03$). CMV disease was similar in both (2 patients in each group). CMV infection appeared at same time after transplant in both groups (at 6 months after transplant), but disease was earlier in ST-group than in LT-group (100 days vs. 144 days respectively), in each case independently of GCV time. Recurrence of CMV disease was not found.

Conclusions: Prolonged oral GCV reduced CMV replication, but not CMV disease rate. The administration of prolonged oral GCV delayed CMV disease. CMV replication was observed during GCV treatment. In presence of GCV prophylaxis, clinical and virological follow-up should be performed more than 120 days (4 months after transplant).

Key words:

CMV disease, oral GCV prophylaxis, antigenemia, PCR

Späte CMV-Erkrankung und/oder Rezidiv bei Nierentransplantatempfängern mit ausgedehnter oraler Ganciclovir-Prophylaxe

¹Nephrology and ²Microbiology I Services, Hospital Universitario Central de Asturias, Oviedo, Spain

Hintergrund und Studienziel: Durch oral verabreichtes Ganciclovir kann einer CMV-Erkrankung wirksam vorgebeugt werden; es wird jedoch – insbesondere bei Langzeit-Prophylaxe – zunehmend eine im späteren Verlauf einsetzende CMV-Erkrankung bei Empfängern von Pankreas-Nierentransplantaten und Knochenmarkstransplantaten beobachtet. Mit der vorliegenden Studie sollte daher geprüft werden, ob eine längere Prophylaxe

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mit oralem Ganciclovir ein spätes CMV-Rezidiv oder eine später auftretende CMV-Erkrankung bei Nierentransplantatempfängern induzieren könnte.

Patienten und Methoden: 64 Nierentransplantatempfänger mit einem Follow-up von 1 Jahr wurden in zwei Gruppen eingeteilt: 27 Patienten in der ST-Gruppe (kurzfristige Prophylaxe), Dauer der oralen GCV-Prophylaxe: 1-2 Monate, und 37 Patienten in der LT-Gruppe (langfristige Prophylaxe), Dauer der Prophylaxe: 3-4 Monate. CMV-Antigenämie und PCR wurden bis 4 Monate nach Transplantation wöchentlich durchgeführt, danach monatlich bis zum Studienende.

Ergebnisse: Nach 4 Monaten war die CMV-Replikation in der ST-Gruppe und in der LT-Gruppe ähnlich (33 % bzw. 32,4 %), nach 1 Jahr wurde sie aber häufiger in der ST-Gruppe als in der LT-Gruppe vorgefunden (77,8 % bzw. 51,4 %, $p=0,03$). Die CMV-Erkrankung war in beiden Gruppen vergleichbar (2 Patienten pro Gruppe). Die CMV-Infektion trat in beiden Gruppen im gleichen Abstand zur Transplantation auf (6 Monate nach Transplantation), die Erkrankung manifestierte sich jedoch in der ST-Gruppe früher als in der LT-Gruppe (100 Tage bzw. 144 Tage), jeweils unabhängig von der GCV-Dauer. Es wurde kein Wiederauftreten einer CMV-Erkrankung beobachtet.

Schlussfolgerungen: Eine ausgedehnte orale GCV-Prophylaxe reduzierte die CMV-Replikation, nicht jedoch die Zahl der CMV-Erkrankungen. Durch längere Verabreichung von oralem GCV konnte die CMV-Erkrankung hinausgezögert werden. CMV-Replikation wurde während GCV-Behandlung beobachtet. Bei GCV-Prophylaxe sollte sich das klinische und virologische Follow-up über einen Zeitraum von mehr als 120 Tagen erstrecken (4 Monate nach Transplantation).

Schlüsselwörter:

CMV-Erkrankung, orale GCV-Prophylaxe, Antigenämie, PCR

Introduction

Cytomegalovirus (CMV) is the most common viral pathogen in renal transplant recipients (1-2). Ganciclovir (GCV) has now supplanted either acyclovir or CMV hyperimmune globulin as the prophylactic therapy of choice for CMV infection and/or disease among transplant recipients (3, 4). Ganciclovir has been given primarily as an intravenous formulation. Oral ganciclovir has also been developed, and it has been demonstrated as effective as intravenous prophylaxis (5, 6). Nevertheless, the ideal anti-CMV prophylactic regimen has yet to be established. On the other hand, in the last years, late-

onset CMV disease in transplant organs is increasingly observed, especially with long-term prophylaxis (7-11). Moreover, some authors have reported that ganciclovir resistance was more common among those patients who received prolonged ganciclovir prophylactic therapy (12, 13). These findings support the hypothesis that the possibility of selecting a mutant virus is enhanced among patients who are exposed to lengthy treatment (14).

The aim of this study was to verify if prolonged administration of oral ganciclovir prophylaxis can induce the appearance of late CMV disease and recurrence in kidney transplants. For this reason, a group of renal transplant re-

cipients with long-term oral ganciclovir prophylaxis and another group with short-term prophylaxis were compared.

Patients and Methods

Patients

From January-2000 to January-2002, 64 consecutive kidney transplant recipients, with a follow-up of one year (336 ± 48 days post-transplant), and who had been treated with oral GCV prophylaxis, were divided in two groups: long-term group (LT-group) included 37 patients who had received oral ganciclovir prophylaxis during 3-4 months (115 ± 19 days after transplant); short-term group (ST-group) included 27 patients treated during 1-2 months (53 ± 12 days after transplant) ($p < 0.0001$). In both groups, CMV replication was studied.

All patients received triple immunosuppression consisting of a calcineurin inhibitor (cyclosporine or tacrolimus), an anti-proliferative agent (mycophenolate mofetil or sirolimus or azathioprine), and prednisone. Moreover, 12 patients from LT-group, and 11 from ST-group received two doses of 20 mg of basiliximab at day 0 and 4 post transplant.

The dose of oral ganciclovir (Cymevene®, Roche, USA) was 1g 3 times daily adjusted to renal function (15). Patients with CMV disease were treated with intravenous ganciclovir for a period of 14 to 28 days. Intravenous CMV hyperimmune globulin was also administered if CMV disease was judged clinically severe.

Clinical and virological (pp65-antigenemia and PCR in peripheral blood leukocytes) follow-up was carried out during the first 12 post-transplant months. Prospective data, with a weekly periodicity during the first 4 months and monthly later, were collected.

In ST-group, 689 samples (25.5 ± 3.5 per patient), and in LT-group, 871 samples (25 ± 3.7 per patient) were collected ($p=0.7$).

Virological Methods

After transplant, patients underwent once weekly testing for CMV viremia

using quantitative blood antigenemia test (CMV-Ag) (16). Leukocytes were separated from peripheral blood by incubation 20' at 37°C. The upper phase was collected and centrifuged 10' at 2000 rpm. Pellet was incubated 1' with sterile water to eliminate erythrocytes. Then, 10⁵ leukocytes were deposited on a slide, air-dried and fixed with formaline. Indirect immunofluorescence with monoclonal antibodies against CMV-pp65 (Sanofi-Pasteur, and Argene-Bio-soft, France) was performed. Quantitative result was informed as positive cells per 10⁵ leukocytes.

Also, CMV single-tube-nested-PCR, with glycoprotein B as target, was performed (CMV-PCR) (17). Leukocytes were treated with proteinase K in order to extract DNA. DNA was added to a reaction mixture with 0.5 pmol of each outer primer (5'-TGA-GGA-ATG-TCA-GCT-TC-3', and 5'-TCA-TGA-GGT-CGT-CCA-GA-3' 5'-TCA-TGA-GGT-CGT-CCA-GA-3'), 1 IU of *Taq* polymerase, 200 µM of dNTPs, and PCR buffer with 2 mM of Mg²⁺ in a total volume of 50 µl. Temperature profile consisted in 5' at 95°C, followed for 25 cycles of 30'' at 95°C, 30'' at 55°C, and 1' at 72°C; with a final extension step during 10' at 72°C. In the second round, 1 IU of *Taq* polymerase, and 25 pmol of each inner primer (5'-CCA-GCC-TCA-AGA-TCT-TCA-T-3', and 5'-TCG TCC AGA CCC TTG AGG TA-3') were added, under the previously described conditions except 30 cycles instead 25. PCR products were analyzed by agarose gel electrophoresis (2% agarose, stained with 0.5 mg/l ethidium bromide).

Definitions

CMV infection was defined as the presence of a positive test (antigenemia or PCR). CMV disease was defined as presence of fever, leukopenia and/or thrombocytopenia, hepatitis, and/or general malaise in absence of other identifiable causes (viral syndrome); and/or pneumonitis, gastroenteritis, and meningoencephalitis (severe CMV disease). CMV disease was considered of late-onset when observed after the first 4 months from the transplant. Recurrence of CMV disease was considered if more than 1 week after conclusion of a successful treatment course, CMV disease was diagnosed.

Tab. 1: Characteristics of renal transplant recipients selected

	ST-group (n=27)	LT-group (n=37)	p
Age (years)	53±3	47±3	0.1
Gender (male/female)	18/9	26/11	0.9
Days in dialysis	1159±276	1770±1775	0.3
Number of transfusions	2.1±0.8	4.5±2	0.3
Days of follow-up	336±48	359±44	0.09
Lymphocytotoxic antibodies			
Pre-transplant	5±13.7	9.3±19.4	0.3
Maximum historic	12.6±24	16.2±24	0.5
Immunosuppressive treatment			
P+CyA+MMF	13	10	0.1
P+CyA+Aza	1	5	0.3
P+CyA+Rapa	1	4	0.3
P+Tac+MMF	12	17	0.9
P+Tac+Aza	0	1	1.0
P+Tac+Rapa	0	0	-
Basiliximab	12	11	0.4
CMV donor/recipients status			
CMV donor + / CMV recipient+	21	22	0.1
CMV donor - / CMV recipient +	4	5	1.0
CMV donor + / CMV recipient -	1	9	0.03
CMV donor - / CMV unknown	1	1	1.0
Acute rejections	2	6	0.4
Corticosteroid-resistant rejections	0	0	

Statistics

Because of small numbers, Fisher's exact test was used to analyze categorical variables. A t- test for paired and unpaired data was used for continuous variables. A p-value of less than 0.05 was considered significant, with Welch correction when the standard deviations differ significantly.

Results

Both LT-group and ST-group were similar in age, gender, time of dialysis, and number of transfusions before transplantation, immunosuppressive treatment, basiliximab administration, pre-transplant and historic lymphocytotoxic antibodies, and days of follow-up (table 1). In LT-group, 9 patients (24.3%) were CMV donor positive/CMV recipient negative vs. 1 (2.7%) from ST-group (p=0.03) (table 1).

CMV Infection

At four months, CMV infection (CMV-Ag+, CMV-PCR+ or both) was de-

tected in 9 patients (33%) from ST-group, and in 12 patients (32.4%) from LT-group. But at 1 year, the patients from LT-group had minor rate of CMV infection than from ST-group (51.4% vs. 77.81%, p=0.03) (figure 1).

These results were also observed when risk patients (D+/R-) were not included: at 4 months, CMV was detected in 9 (34.6%) of 26 patients from ST-group, and in 10 (35.7%) of 28 from LT-group; at 1 year, CMV was detected in 20 patients (77%) from ST-group, and in 14 (50%) from LT-group (p=.05)

During GCV prophylaxis, CMV replication was detected in 2 (7.7%) patients by PCR at 21 and 23 days post-transplant, in ST-group. In LT-group, CMV replication was present in 13 (36.1%) at a mean of 48.3±41.4 days post-transplant as by PCR (11 patients) as CMV-Ag (4 patients), significantly more frequently than ST-group (p=0.014).

After GCV prophylaxis, CMV infection was present in 20 (74%) patients in ST-group, and in 10 (27.7%) renal transplant recipients in LT-group (p=0.011). Whereas CMV-Ag was detected in similar number of cases in both groups (7 in ST-group, and 4 in LT-group, p=0.6), CMV-DNA was detected more

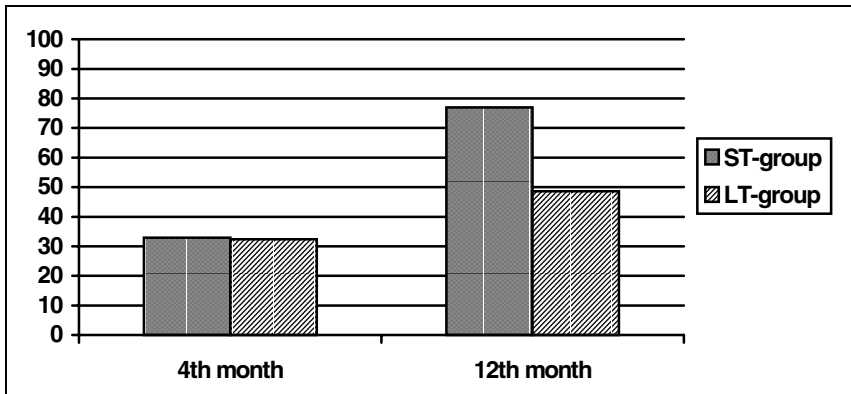


Fig. 1: Evaluation of CMV infection in ST-group and in LT-group, at two different controls after transplant.

frequently in ST-group than in LT-group (15 vs. 6 respectively, $p=0.001$). CMV replication was detected at a mean of 185.2 ± 98.2 days post-transplant (range 38-322) in ST-group, and at a mean of 192.2 ± 75 days post-transplant (range 99-335) in LT-group, $p=0.7$. According GCV prophylaxis, in ST-group, CMV appeared 127.3 ± 95.5 days after discontinuation of GCV, and at 84.8 ± 76.78 days in LT-group.

CMV Disease

Two patients from LT group and two from ST group (6,2% of the total) developed CMV disease ($p=1.0$). The disease always appeared after the end of the prophylaxis: in the 2 patients of ST-group, at days 91 and 106 from renal transplant (62 and 76 days after the end of GCV); in the 2 patients of LT-group at 143 and 144 days post-TR (93 and 18 days post-GCV). Both diagnosing methods, antigenemia and PCR, preceded to the symptoms. Only one patient had D+/R- status. The disease was mild in 3 of the 4 patients. A patient from ST-group had a severe gastrointestinal CMV disease. All patients responded to the treatment with intravenous ganciclovir. There were not recurrences.

Discussion

The natural history of CMV disease associated with solid organ transplantation has been modified as a result of the widespread use of antiviral prophylaxis. Intravenous GCV has been demon-

strated more effective than other antiviral drugs (5, 6, 18). Oral GCV has also been demonstrated as effective as intravenous prophylaxis. Prolonged treatment with oral GCV, despite less than 10% bioavailability, is potent prophylactic therapy for CMV disease. Several randomized trials have documented that the rate of CMV disease during treatment with 3 to 4 months of oral GCV in doses as low as 250 mg twice daily is less than 5% (5, 6, 7, 19). But current prophylactic approaches vary widely among different transplant programs and guidelines to use GCV prophylaxis have yet not been established.

In this study, we compared patients with ST-prophylaxis, and LT-prophylaxis. In a classical analysis at 4 months from transplant we could check that CMV replication was similar in both groups, but at 1 year, CMV replication was lower in LT-group (similar results if we analyze the patients without D+/R- status). However, we could not avoid CMV disease, which occurred later than we expected, in agreement with previous works (20). We found CMV disease at 100 days after transplant in ST-group, and at 144 days in LT-group. These results have been reported by others authors, who demonstrated that patients with prolonged periods of GCV prophylaxis developed late CMV disease in renal transplant as well as bone marrow transplant recipients (10, 11, 21). CMV disease occurred in 2 patients of each group, in spite of LT-group comprising more risk patients (D+/R-).

In some cases, recurrence after a first episode of CMV disease has been described (11). In this study, recurrence was not observed. Nevertheless, CMV

replication was detected as long as 8-9 months after transplant (independent of ST or LT protocol). Then, clinical and virological follow-up should comprise this period.

The use of prolonged therapy with GCV could cause several problems. Turgeon et al. demonstrated that oral GCV following intravenous GCV slightly reduced the overall rate of recurrent CMV disease and/or viremia, but it still did not adequately prevent CMV recurrence in patients who are at risk of primary infection prior to transplant. Of particular concern, 2 patients with primary infection treated with this regimen developed ganciclovir-resistant recurrent disease (13). Only one of our 4 patients was CMV donor-positive and CMV recipient-negative, and none received anti-lymphocytic drugs. According GCV-resistant strain of CMV, reports that the use of ganciclovir may select for drug-resistant CMV have been increasing (22, 23, 24). However, Gilbet et al. did not find CMV UL97 mutations in 10 allogeneic stem cell transplant recipients after mean GCV exposure of 31 days (25). In a previous study in our hospital, we could observe that IC_{50} of GCV to CMV strains, was higher in patients under prophylaxis regimen, and one of them caused a resistant strain (24).

In this study, CMV replication was just detected by PCR in 2 patients in ST-group during GCV regimen, but CMV was present in 13 (36%) from LT-group (4 of them by antigenemia). Then, a rising load or CMV level may imply suboptimal suppression and the potential for emergence of GCV resistance. These findings support the hypothesis that the possibility of selecting a mutant virus is enhanced among patients who are exposed to lengthy treatment. This indicates a need for increased vigilance for antiviral resistance, especially as oral antiviral preparations are used for prolonged periods, either for prophylaxis or as treatment.

An approach to avoid these problems and unnecessary prophylaxis in patients who are at no increased risk for CMV-associated morbidity and death, could be pre-emptive therapy. Early therapy given in this manner is dependent on a laboratory marker or patient characteristic that identifies the subgroup of individuals at an increased risk for disease at a time when antimicrobial intervention would be maximally effective

in aborting the impending disease process (26). For this purpose, antigenemia or PCR tests could be good markers (20, 27). In our data, antigenemia as well as PCR preceded CMV disease.

Whether pre-emptive therapy or universal prophylaxis is the optimal approach against CMV remains unresolved (28). Last year, new antiviral drugs against CMV were presented. Valacyclovir, a prodrug of acyclovir, was recently shown to be effective compared with placebo (29). Valganciclovir, a ganciclovir prodrug with increased oral bioavailability, has demonstrated to be as effective as GCV in the prophylaxis of the CMV disease (30). The effect of these new drugs at the time of appearance and the recurrence of the disease are well documented.

In summary, the prolonged administration of oral ganciclovir decreased CMV infection and delayed CMV disease in renal transplant patients. The rate of disease is very low as much in the group with long-term prophylaxis like in the group of short-term prophylaxis. Because late CMV infection and disease was detected, clinical and virological follow-up must be performed more than 120 days. CMV replication during GCV prophylaxis could cause CMV resistant-mutants. The systematic administration of prophylaxis could change the natural history of the disease by CMV in the organ transplants. Then, this approach should be revised.

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Ernesto Gómez
Servicio de Nefrología
Hospital Universitario Central
de Asturias
C/ Celestino Villamil, s/n
E-33006 Oviedo
Asturias, Spain
E-mail: egomez@hcas.sespa.es