ORIGINAL ARTICLE

Pre-emptive oral ganciclovir can reduce the risk of cytomegalovirus disease in liver transplant recipients

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A cohort of 65 liver transplant recipients was prospectively monitored with qualitative polymerase chain reaction (PCR) in plasma. The first 25 patients did not receive prophylaxis. From a consecutive group of 40 recipients, 11 high-risk patients donor CMV-seropositive/receptor CMV-seronegative (D+/R–), persistent CMV replication) received pre-emptive oral ganciclovir (1000 mg three times daily), when a marker of risk was identified, until day 90. The overall incidence of cytomegalovirus (CMV) disease at six months was 20% (five of 25 patients) in the non-prophylaxis group and 2.5% (one of 40 patients) in the group treated with pre-emptive oral ganciclovir (relative risk, 0.11; 95% confidence interval; 0.01–0.96; $P = 0.04$). The PCR sensitivity for detecting CMV disease was 80%, the specificity was 90%, and the positive and negative predictive values were 66% and 95%, respectively. Adverse events, graft rejection and survival were similar between groups. We conclude that pre-emptive oral ganciclovir in high-risk patients can reduce the risk of CMV disease.

Keywords CMR, oral ganciclouir, pre-emptive, transplantation

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INTRODUCTION

Cytomegalovirus (CMV) is an important opportunistic pathogen following liver transplantation [1,2]. Active CMV replication causes various clinical infectious syndromes; CMV has immunosuppressive properties and has been associated with vanishing bile duct syndrome (though not in all studies) [3]. Several prophylactic strategies have been developed to reduce the incidence of CMV disease. Pre-emptive therapy involves the administration of a highly effective antiviral agent to a subgroup of patients who are at risk of disease based on a clinical donor CMV-seropositive/receptor CMV-seronegative (D+/R–) or laboratory risk marker. Pre-emptive therapy can be used for patients with CMV replication on the assumption that persistent replication is a reliable predictor of disease [4]. CMV DNA can be detected in the sera or plasma of liver transplant recipients using the polymerase chain reaction (PCR) prior to onset of symptomatic CMV infection, and could be a potential marker for viral replication and pre-emptive therapy [5,6].

Effective and safe oral antiviral agents need to be developed for pre-emptive therapy to become a promising approach. One prospective, randomized, placebo-controlled study has shown universal oral ganciclovir to be effective in preventing CMV infection and disease following liver transplantation [7]. Nevertheless, its efficacy in aborting ongoing viral replication is not yet known.

This paper reports a study of the clinical value of PCR detection of viral DNA and the efficacy and safety of pre-emptive oral ganciclovir in aborting CMV disease.

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PATIENTS AND METHODS

Study design

Eligible patients were over 12 years old and were undergoing primary liver transplantation.

Recipients of multiple organs were excluded. The first cohort of patients received no prophylaxis and they were monitored by means of CMV DNA detection in plasma. Depending on the results obtained from these patients, a second cohort of patients received pre-emptive therapy with oral ganciclovir when a marker of risk was identified (D+/R– match, DNA CMV detected in plasma in two consecutive weeks) until day 90. Patients not treated were: those unable to take oral medication; and those with a neutrophil count <1000 cells/μL, platelet counts $<25\,000$/μL, or serum creatinine levels >300 μmol/L on entry. The dosage of oral ganciclovir was adjusted for impaired renal function as follows: patients received 3000 mg/day if creatinine clearance was ≥ 50 mL/min, 1000 mg once a day if creatinine clearance was 25–49 mL/min, 500 mg once a day if creatinine clearance was 10–24 mL/min, and 500 mg three times a week if creatinine clearance was less than 10 mL/min. Oral ganciclovir capsules were taken 30 min after a meal. Where CMV disease was diagnosed during the study period, the patient was routinely treated. Prophylactic aciclovir was prohibited. Patients started selective oral decontamination when transplantation was indicated, continuing until hospital discharge. Perioperatively, patients were given ampicillin (1 g/6 h) and ceftazidime (1 g/8 h) until 48 h after transplantation. *Pneumocystis carinii* prophylaxis consisted of 500 mg sulfadoxin/25 mg pyrimethamine (Fansidar, Roche, Switzerland) once a week, as previously described [8].

Monitoring

Patients were routinely assessed on a daily basis over the first two weeks, and then weekly until the end of the third month. Assessment consisted of clinical examination, determination of blood counts, creatinine, ions, liver enzymes, and cyclosporin A/tacrolimus levels, and urine analysis (sediment, ions and creatinine). Blood surveillance viral cultures (shell vial) were performed every two weeks for the first month and then at monthly intervals until the third month. They were repeated when clinically indicated. Detection of CMV DNA in plasma was done at weeks 2, 3, 4, 5, 6, 7, 8, 10 and 12 after transplantation. Patients were also assessed after six months.

Immunosuppressive regimen

Maintenance immunosuppression consisted of standard triple therapy with cyclosporin or tacrolimus, steroids and azathioprine. Rejection was diagnosed histologically. Liver biopsy specimens were obtained whenever hepatic dysfunction occurred. Rejection episodes were treated with three doses of 1 g of methylprednisolone and/or an increase in oral prednisone that was reduced to baseline values in five–seven days.

Definitions

CMV infection was considered to exist when the virus was isolated either in blood or in a biopsy. CMV disease was considered to exist when the culture or histologic evidence of CMV infection was accompanied by consistent symptoms. Viral syndrome was defined as persistent fever and leukopenia, with or without anemia and thrombopenia, which could not be attributed to other causes in a patient with evidence of infection according to culture. Organ disease was defined as symptomatic dysfunction with histologic evidence of infection (definitive diagnosis) or viral isolation in a biopsy culture without histologic evidence (probable diagnosis). CMV replication was considered 'persistent' when CMV DNA was detected in plasma over two consecutive weeks.

Plasmatic PCR procedure (nested PCR)

Plasma samples were maintained at $-20\,^{\circ}$C until processing for a period ranging from one to five-days. EDTA was used in plasma collection. Nucleic acids were extracted from plasma with 0.1 M NaOH (v/v) for 1 h at 37 °C and neutralized with 0.1 M HCl. The solution was treated with phenol–chloroform and chloroform–isoamyl alcohol. The primers used in the nested PCR assay were taken from the fourth exon of the CMV IEA1 gene (Towne strain). The outer primer pair was 5′-CAAGCGGCCTCTGATAACCAAGC-3′ complementary to the coding DNA strand nucleotides 731–753, and 5′-CTCTTCCTCTGGGGCAACTTC-CTC-3′ complementary to the non-coding DNA nucleotides 1167–1144. These primers amplified a 438-bp fragment of DNA. The inner pairs of primers were 5′-GCCGATCCTCTGAGAGTCTG-CTCTC-3′ complementary to coding strand nucleotides 829–851, and 5′-CAGCCACAATTA-

CTGAGGACAGAGG-3′ complementary to non-coding DNA nucleotides 1019–994. These primers amplified a 190-bp fragment of DNA. Reaction mixtures consisted of 5 μL of plasma, 20 pmol (0.4 μM) of each oligonucleotide primer, 1.25 U of the enzyme *Taq* polymerase (Perkin Elmer Cetus, Norwalk, CT, USA), 200 μM (each) of doxynucleotide triphosphates (dATP, dCTP, dGPT and dTTP), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$, and 1% Triton X-100, to a total volume of 50 μL in a microcentrifuge tube. In the first amplification, tubes were subjected to 30 cycles (94 °C for 1 min, 65 °C for 1 min, 72 °C for 1 min + 10 s) in a DNA thermocycler (Perkin Elmer Cetus). In the second amplification, 5 μL of amplified solution was used, employing the same reaction mixture. In this case, samples were subjected to 40 cycles (94 °C for 1 min, 65 °C for 1 min, 72 °C for 1 min + 10 s). Amplified PCR products were electrophoresed on an agarose gel and visualized with ethidium bromide under ultraviolet light. For each PCR batch, three tubes containing the reaction mixture but no target DNA were run. All tubes containing the reaction mixture but no target DNA yielded negative results.

Statistical analysis

The Student *t*-test was used for quantitative data and the chi-square test for qualitative data. The primary endpoint in this study was time to development of CMV disease during the six-month study period. Secondary endpoints were patient survival, acute graft rejection, and graft loss. Kaplan–Meier product limit estimates of event rates were calculated at six months. The Cox proportional hazards model was used to estimate relative risk for the treatment effect. Logistical regression was used to analyze intergroup differences in graft rejection and graft loss.

RESULTS

Demographics

Between November 1995 and December 1997, 80 patients received a liver transplant at this center. Fifteen patients were excluded from the study: ten patients had exclusion criteria (unable to take oral medication, three patients; neutrophil count <1000 cells/μL, two patients; platelets counts <25 000/μmol, two patients; serum creatinine >300 μmol/L, three patients), four patients were not able to comply with the study protocol, and one patient was unlikely to survive for more than 24 h. Complete six-month data were thus available for 65 patients (Figure 1). A first cohort of 25 patients not receiving viral prophylaxis was assessed with qualitative PCR in plasma. A second consecutive cohort of 40 patients was treated with pre-emptive oral ganciclovir in D+/R– patients and in patients with positive PCR in plasma in two consecutive weeks. The two cohorts were well matched for sex, age, primary liver disease and immunosuppression (Table 1). In the first cohort of 25 patients, 23 recipients were CMV seropositive (R+) but received CMV-seropositive or -seronegative donor organs; two recipients were CMV seronegative but received CMV-seropositive donor organs (D+/R–). In the second cohort of 40 patients, 38 recipients were R+, one patient was D+/R–, and one patient was CMV seronegative, but the donor's CMV serology was unknown.

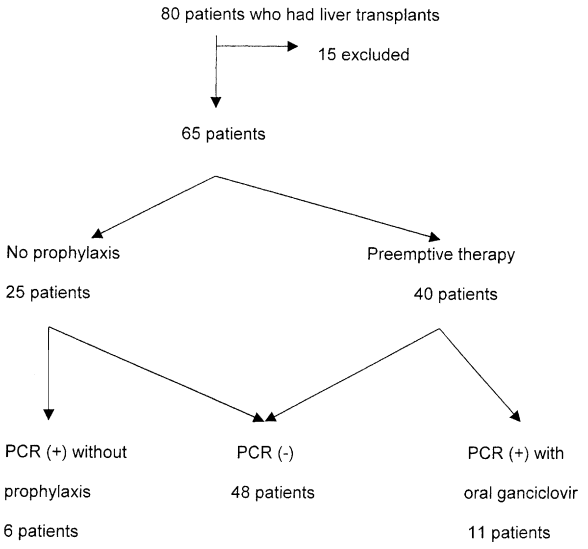

Figure 1 Trial profile.

Ability of persistent replication assessed by qualitative PCR to predict CMV disease

The value of persistent CMV replication as a predictor of CMV disease was assessed by 2 × 2 table analysis (Table 2). Of the five patients with CMV disease, four (80%) also displayed persistent replication. One patient with CMV hepatitis had never presented CMV replication in plasma. This

Table 1 Patient demographics, primary liver disease and CMV serostatus

	'Wait and treat' ($n = 25$)	Pre-emptive oral ganciclovir ($n = 40$)	*P*
Mean age (years)	48 ± 12	48 ± 13	NS
Sex male	17 (68%)	27 (67%)	NS
Primary liver disease			
Alcohol	8 (32%)	5 (12.5%)	NS
Hepatitis C virus	8 (32%)	20 (50%)	
Primary biliary cirrhosis	1 (4%)	2 (5%)	
Fulminant hepatic failure	2 (8%)	1 (2.5%)	
Hepatitis B virus	2 (8%)	6 (15%)	
Other	4 (16%)	6 (15%)	
Pretransplant CMV serology			
Receptor positive	23 (92%)	38 (95%)	NS
Donor positive/receptor negative	2 (6%)	1 (2.5%)	
Donor unknown/receptor negative	0	1 (2.5%)	

NS, not significant; Student *t*-test and chi-square test.

Table 2 Number of liver transplant recipients from the first cohort ('wait and treat' policy) with CMV disease as a function of persistent CMV replication

	CMV disease		
	Present	Absent	Total
Persistent replication			
Present	4	2	6
Absent	1	18	19
Total	5	20	25

Sensitivity = 4/5 = 80%; specificity = 18/20 = 90%; positive predictive value = 4/6 = 66%; negative predictive value = 18/19 = 95%.

analysis revealed a sensitivity of 80%, a specificity of 90%, and positive and negative predictive values of 66% and 95%, respectively. One D+/R– patient presented a second positive PCR at the same time as CMV hepatitis. Four R+ patients displayed persistent second positive PCRs for a minimum of seven days (mean, 21; median, 14) prior to the onset of CMV disease. The other D+/R– patient died on day 34, preventing adequate follow-up. Excluding the two D+/R– patients, analysis revealed a sensitivity of 75%, a specificity of 89%, and positive and negative predictive values of 60% and 94%, respectively.

Clinical outcome of prophylaxis with oral ganciclovir

We first analyzed the risk of CMV disease associated with both policies; 'wait and treat' compared with pre-emptive oral ganciclovir (Table 3). The incidence of CMV disease at six months was 20% (5/25 patients) in the group without prophylaxis ('wait and treat' policy) and 2.5% (1/40 patients) in the group treated with pre-emptive oral ganciclovir (RR = 0.11, 95% CI = 0.01–0.96,

Table 3 Incidence of cytomegalovirus disease

Regression model	CVM disease at six months	Relative risk (95% CI)	*P*
Global policy			
'Wait and treat' ($n = 25$)	5 (20%)	Reference	–
Pre-emptive oral GCV ($n = 40$)	1 (2.5%)	0.11 (0.01–0.96)	0.04
CMV replication			
Replication without oral GCV ($n = 6$)	4 (66.6%)	Reference	
Replication with oral GCV ($n = 11$)	0 (0%)	0.05 (0.01–0.25)	< 0.01

CMV, cytomegalovirus; GCV, ganciclovir; NS, not significant.
Kaplan-Meier estimates and Cox proportional hazards regression.

$P = 0.04$). Eleven patients received oral ganciclovir in the pre-emptive group during a median of 50 days (range, 37–65). Oral ganciclovir was administered with a median delay of three days (range, one–five days) after the second positive PCR was observed. All patients were PCR negative after three weeks of treatment.

In the 'wait and treat' cohort, five patients developed CMV disease; viral syndrome, two patients, enteritis, one patient, and hepatitis, two patients. Before transplantation, four patients were D+/R+ and one patient was D+/R– (hepatitis). All five patients but one had persistent CMV replication (two consecutive positive PCRs). One D+/R– patient presented a second positive PCR at the same time as CMV hepatitis. The patient displaying CMV disease from the pre-emptive therapy group was CMV seropositive before transplantation and he never received oral ganciclovir, since plasma PCR was always negative. The patient presented local reactivation of CMV in the allograft (hepatitis). Two of the 48 patients (4.2%) with negative PCR CMV in all measurements developed CMV disease.

All patients with CMV disease responded to a single course of intravenous ganciclovir. None of these patients developed recurrent CMV disease. Between the three-month and the six-month follow-up visits, no patients from either group developed CMV disease.

The efficacy of oral ganciclovir in preventing CMV disease was also analyzed as a function of persistent CMV replication. Oral ganciclovir was associated with a significant reduction in rate of CMV disease in PCR-positive patients receiving oral ganciclovir (11 patients) as compared with non-treated PCR-positive patients (six patients). The incidence of CMV disease at 6 months was 66.6% (4/6 patients) in PCR-positive patients not receiving oral ganciclovir, compared with 0% (0/11 patients) in PCR-positive patients treated with oral ganciclovir. Ganciclovir prophylaxis produced an overall reduction of CMV disease in patients with persistent replication (relative risk (95% CI), 0.05 (0.01–0.25), $P < 0.01$).

During the six-month study period, one patient (9%) developed symptomatic herpes simplex virus infection in the group of patients with positive PCR treated with oral ganciclovir; this occurred two weeks before oral ganciclovir was indicated. One patient (16.6%) in the group of positive PCR without prophylaxis and six patients (12.5%) in the group of negative PCR had herpes simplex virus disease.

No intergroup differences were recorded in terms of either the incidence of acute graft rejection or graft loss during the six-month follow-up period.

Adverse events during oral ganciclovir treatment

The most common adverse events during the six-month study period were diarrhea, anemia and elevated serum creatinine. These occurred with similar frequency in the 'wait and treat' group and in the pre-emptive ganciclovir group: diarrhea, 28% (7/25 patients) versus 30% (12/40 patients); anemia, 60% (15/25 patients) versus 62.5% (25/40 patients); elevated serum creatinine ($\times 2$), 16% (4/25 patients) versus 17.5% (7/40 patients).

Survival

Mortality rates at the end of the sixth month were 16% (four of 25 patients) in the non-prophylaxis cohort and 10% (four of 40 patients) in the cohort treated with pre-emptive oral ganciclovir, but differences were not significant. One of the six patients (16.6%) developing positive plasma PCR and not treated with oral ganciclovir died, but death was not directly attributable to CMV disease.

DISCUSSION

The first part of this study was designed to determine whether persistent CMV replication, defined as positive plasmatic PCR in two consecutive weeks, could be used as a marker for pre-emptive therapy. Our results suggest that persistent replication is a marker of the risk of CMV disease and that plasmatic PCR provides a high degree of sensitivity and specificity for predicting the onset of CMV disease. It may be a useful laboratory marker for pre-emptive therapy.

Some authors have detected CMV DNA in plasma even when cell-free virus cannot be detected in plasma by standard culture techniques, reflecting the extremely high sensitivity of PCR for the detection of small amounts of extracellular free virus or viral fragments [5,9,10]. It gives rise to false positives and low specificity when only one sample is taken into account. Other

authors have reported a high positive predictive value of one positive PCR for predicting CMV disease in transplant patients using the Cobas Amplicor CMV Monitor PCR test [5]. Nevertheless, we have observed that intermittent DNAemia in asymptomatic patients accounted for false positives [10]. This could reflect a higher sensitivity of our PCR test, which could detect replication in clinically insignificant sites, such as the genitourinary tract; in most cases, positive results are followed in later weeks by negative results. The data obtained here suggest that specificity for predicting CMV disease is high, if we consider only those patients with positive results in two consecutive weeks as being at risk. Although viral load quantification has been proposed as an alternative to increased specificity, a study has shown that quantitative PCR from peripheral blood provided no additional advantage to qualitative PCR for predicting CMV disease [9]. Although the detection of persistent CMV replication using PCR in plasma was not followed by disease in one of every three patients (positive predictive value of 66%), the relative risk of CMV disease related to non-treated persistent replication was extremely high. Furthermore, with a negative predictive value of 95%, the chance of a patient having CMV disease without CMV persistent replication was extremely low. Therefore, detection of persistent DNAemia in plasma is a good marker for guiding pre-emptive therapy.

CMV-naive patients (D+/R–) may represent an exception for early identification of patients who will later develop CMV disease. A CMV-naive patient in the 'wait and treat' group simultaneously developed CMV disease and the second positive PCR. It has recently been shown that in D+/R– liver transplant patients not receiving antiviral prophylaxis, all viral load levels are sufficient for identifying patients developing the disease [11], raising the question of whether surveillance of CMV replication is indicated in CMV-naive patients. Thus, D+/R– serostatus provides prognostic information because it identifies a subgroup of patients who will develop high CMV loads post-transplant [4].

For a prophylactic regimen to be widely applicable, it must be effective, practical, safe and inexpensive. A recent study has shown that oral ganciclovir at a dosage of 3 g/day until day 98 post-transplant is a safe and effective method for preventing CMV disease after liver transplantation [7]. Oral ganciclovir was effective and safe in all the subgroups analyzed. This was the first study to demonstrate a significant benefit for antiviral prophylaxis in the D+/R– subgroup or in liver transplant recipients, who are at the greatest risk from CMV disease. Two potential concerns regarding universal prophylaxis with oral ganciclovir are the possible emergence of drug-resistant viral strains and its economic cost. Optimization of pre-emptive therapy requires further development of effective oral drugs that may be used in asymptomatic outpatients when the laboratory marker is positive.

The second part of our study was designed to determine whether pre-emptive oral ganciclovir was effective and safe for preventing CMV disease. Oral ganciclovir may reduce the incidence of symptomatic CMV infection without severe side effects, avoiding the cost and risk of universal prophylaxis. These results suggest that viral replication may be aborted in seropositive receptors before CMV disease is achieved, by using oral ganciclovir. Recommended target serum concentrations of ganciclovir obtained with the oral formulation are 0.5–1.0 μg/mL, which is within the IC_{50} range of most CMV isolates [12], and which would effectively abort the disease process when a risk patient is identified rapidly. Nevertheless, ganciclovir levels were not determined in this study. Since D+/R– serostatus is a known risk factor of CMV disease, we decided to initiate oral ganciclovir immediately after transplantation in this patient subgroup. The only D+/R– patient included in this second cohort never developed viral replication or CMV disease during the follow-up period.

The primary toxicity of ganciclovir is myelotoxicity [13,14]. Whenever oral ganciclovir was indicated, it was not associated with severe myelotoxicity. The drug was well tolerated without significant side effects.

Based on the results obtained here, we believe that the protocol used offers several advantages over universal prophylaxis with oral ganciclovir. It significantly reduced the use of the drug, since D+/R– accounted for few patients, and CMV-positive receptors with persistent replication corresponded to only one of every three or four patients. The emergence of resistance is always a cause for concern [15,16], particularly in light of a recent article showing that application of the pre-emptive therapy strategy in D+/R– patients

notably increases the risk of generating ganciclovir-resistant CMV [17]. The reduction in medication costs will probably be cancelled out by an increase in viral surveillance costs.

Similar results have been observed in another study, in which pre-emptive oral ganciclovir was guided by antigenemia [18]. An interesting difference between the two studies is that, whereas in the present study oral ganciclovir (1000 mg three times daily) was administered until week 12, the other study used a loading dose (2000 mg three times daily) for two weeks, followed by a standard dosage (1000 mg three times daily) for four weeks. Since all of our patients were PCR negative after three weeks of treatment, we suggest that the loading dose can be avoided.

This study has clear limitations. First, the sample size was small and the follow-up was short. Second, this was not a randomized study. For these reasons, it can only be considered as a prospective pilot study. A prospective, randomized and controlled trial comparing universal oral ganciclovir with the approach adopted here is required in order to establish its value. Future studies should also address the potential value of pre-emptive therapy using other available anti-CMV oral drugs (valganciclovir, 1263W94, etc.) and the risk of generating ganciclovir-resistant CMV strains.

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The human experimentation guidelines of the Hospital Universitario Reina Sofia were followed.

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