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Failure of acyclovir to prevent cytomegalovirus infection in renal allograft recipients*

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Abstract. Cytomegalovirus (CMV) is the most common opportunistic pathogen following renal transplantation and remains a major concern in transplantation centers owing to its high morbidity and impact on renal allografts. Pending more effective antiviral drugs, efforts have been directed toward prevention strategies. We conducted a retrospective analysis to evaluate the efficacy of various prophylactic options used at our institution during the period April 1986 to August 1990. All CMV-negative patients with CMV-negative kidneys (D– R–) received screened, CMV-negative blood products ($n = 19$). CMV-specific immunoglobulins (CMV Ig) were used in 6 patients at increased risk for primary CMV infection and acyclovir was administered to 21 patients at an initial intravenous dose of 5 mg/kg body weight; then oral doses of 800–3200 mg per day were given according to the patients' estimated creatinine clearance. Thirty-two patients did not receive any CMV prophylactic treatment and served as controls. CMV monitoring of the patients during the first 6 months after transplantation showed an overall infection and disease rate of 81 % and 38.1 %, respectively, in the acyclovir-treated group. Compared with controls, the incidences of infection and disease were higher in the acyclovir-treated patients, with a significant difference for CMV infection ($P = 0.002$, generalized Wilcoxon test). Only 1 of the 19 D– R– patients presented with CMV infection. CMV Ig-treated patients tended to have less severe disease without any apparent reduction in infection incidence. Given the high rate of infection in patients at risk, we infer that high-dose acyclovir does not prevent CMV infection in our setting of renal transplantation. We advocate the use of screened, CMV-negative blood products in D– R– patients.

Key words: Cytomegalovirus, acyclovir – Acyclovir, prevention of CMV – Prevention of CMV, acyclovir – Kidney transplantation, acyclovir

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Introduction

In the era of cyclosporin therapy, cytomegalovirus (CMV) still emerges as the most common opportunistic pathogen following bone marrow or solid organ transplantation. The ubiquity of CMV infection among renal transplant patients has been well documented, with 60 %–96 % of these patients demonstrating evidence of infection in the 1st year after transplantation [14, 18]. The spectrum of CMV infection ranges from asymptomatic viral excretion or seroconversion to invasive disease of the gastrointestinal tract, liver, eyes, and lungs, with life-threatening interstitial pneumonia being the most fearful event.

Recently, a new antiviral drug, ganciclovir, has been found to be effective in the treatment of CMV disease and has improved survival in patients with this disease [4, 12, 22]. Nevertheless, control over CMV replication still remains a great concern in transplantation centers, owing to its high morbidity and impact on renal allografts [8, 17, 19]. Pending more effective antiviral drugs, efforts are still being focused on prevention strategies.

The demonstration that primary CMV infection among seronegative patients with seronegative kidney donors can be eliminated by the use of screened, seronegative blood products has been well documented [1]. However, because of various epidemiological factors, including recipient and donor age and previous blood transfusions, seronegative patients with seronegative donors comprise a minority of the renal transplant population. Moreover, matching for CMV serology among recipient-donor pairs would cause unnecessary delays for transplantation. This strategy would also be unfair to the seropositive recipients who would be given only seropositive kidneys since reinfection by the donor kidney is usually more frequently symptomatic than endogenous reactivation [11].

Other prophylactic measures for CMV infection in kidney transplant recipients have yielded mixed results. Interferon alpha was not found to be effective and has been associated with a high frequency of graft dysfunction [10].

Passive and active immunizations with CMV-specific immunoglobulins (CMV Ig) and by the Towne strain vaccine have provided conflicting results: they seem to protect against severe primary CMV disease in seronegative recipients but do not reduce the infection rate [3, 15, 16, 23]. More recently, acyclovir, given orally to kidney transplant recipients, reduced the rate of infection from 61 % to 36 % when compared to placebo. On the basis of these results, high-dose acyclovir was used prophylactically in our center in 21 kidney recipients at risk for CMV infection and disease. We report our experience with this agent, comparing the results obtained with that from a control group who had received no prophylaxis. Results obtained with other preventive measures are also given.

Patients and methods

Study population

From April 1986 to August 1990, 88 renal transplantations were performed at our institution. Records from all of the transplant recipients were carefully reviewed and data collected for analysis. Except for two patients who received grafts from living related donors, all patients were transplanted with cadaveric kidneys. During that period different options for CMV prophylaxis were used and these changed with time, according to what was reported in the literature. Data from ten patients were excluded from final analysis either because of immediate postsurgical death ($n = 1$) or because of early transplantectomy or permanent graft dysfunction ($n = 9$). The 78 remaining patients could be subdivided into four groups according to the CMV prophylaxis they received.

Group 1. This group was comprised of all seronegative recipients of seronegative donors (R– D–), a group at low risk for CMV infection. Special efforts were made to ensure that they received only screened, CMV-seronegative blood products. There was a total of 19 patients in this group.

Group 2. CMV prophylaxis in this group consisted of intravenous CMV Ig. It was a small group of six patients who were considered as being at increased risk for primary CMV infection, i.e., seronegative recipients of seropositive kidney donors (R– D+). This regimen was used shortly before the use of high-dose acyclovir.

Group 3. Patients in this group received acyclovir (Zovirax) as prophylaxis for CMV infection. This strategy was initiated after publication of the randomized, controlled study conducted by Balfour and colleagues demonstrating the efficacy of high-dose oral acyclovir in preventing CMV infection [16]. Twenty-one patients comprised this group.

Group 4. A total of 32 patients, transplanted earlier, did not receive any prophylactic treatment and were assigned to this group to serve as controls. Data from this group could be compared to those of group 3 since they were of comparable size and all patients had similar clinical and virological management.

Treatment protocols

The immunosuppressive regimen for allograft tolerance was constant throughout the study period. All patients received prophylactic antilymphocyte or antithymocyte globulins and triple maintenance therapy with cyclosporin, azathioprine, and prednisolone. Rejection episodes were treated with methylprednisolone boluses and corticosteroid-resistant rejections with OKT3 ($n = 2$).

CMV Ig globulin (Transfusion Centre), Lille, France) was administered intravenously at a dose of 500 units/kg body weight, starting within 72 h following surgery and weekly thereafter for 6 weeks. It was then reduced to 50 units/kg body weight per week for another 6 weeks.

Acyclovir was given intravenously at an initial dose of 5 mg/kg body weight within the 6 h prior to surgery. Then, oral doses of 800 mg were given every 6, 8, or 24 h if the creatinine clearance was greater than 25 ml/min, between 10 and 25 ml/min, or less than 10 ml/min, respectively. Patients on hemodialysis received 800 mg of acyclovir every 12 h. Prophylaxis was intended to last for 3 months. Acyclovir was discontinued whenever ganciclovir was used for treatment of CMV disease.

Clinical management and virological studies

Whenever available, serum from donors was screened for CMV antibody with the latex agglutination assay and, in all instances, results were obtained before surgery. Confirmation of the CMV serology status was then obtained using an ELISA test. All recipients were screened for CMV antibody using an ELISA test before transplantation. No special effort was made to match donor-recipient pairs according to CMV serology. All patients were followed from the time of transplantation and were seen daily during the hospitalization period (mean 21 days), biweekly for the following 2 months, then weekly until the 6th month, and twice monthly thereafter for the 1st year.

Urine and blood specimens for viral isolation were routinely collected weekly from the 1st month up to 3 months after transplantation and monthly thereafter until the 6th month. Additional virological studies were done if CMV disease was suspected. The presence of CMV was determined using both a rapid culture (detection of early antigen fluorescent foci) and a standard culture (detection of cytopathic effect on human fibroblasts) technique. All cultures were read by the same virologist who was blind as to the patients' treatment.

Definitions

CMV infection was defined as the isolation of the virus from any site and CMV disease as the occurrence of one or more symptoms attributable to CMV concurrently with CMV isolation.

Each patient's record was reviewed in retrospect for the presence of fever, respiratory symptoms, gastrointestinal tract disorders, transplantectomy, pancreatitis, or superinfection. The severity of CMV disease was then scored according to the six criteria used by the Minnesota Health Sciences Center, described elsewhere [14].

Time of onset of infection or disease was the time between transplantation and the first CMV-positive culture or the first symptom, respectively.

Statistical analysis

Values are expressed as means ± standard deviation (SD). The main objective of the analysis was to evaluate the efficacy of acyclovir in preventing CMV infection and disease. Since group 3 (acyclovir-treated) and group 4 (controls) were comparable in size, we decided to compare data from these two groups. Student's unpaired t-test and Pearson's chi-square (χ^2) test were used for comparison between groups.

The probability of surviving infection and disease in groups 3 and 4 was computed for the first 6 months after transplantation according to Kaplan-Meier's method and was compared using the generalized Wilcoxon test.

A significant difference was defined as a P value less than 0.05.

Table 1. General characteristics of patients enrolled in the study

Characteristic	Values	
	Mean (±SD)	Range
Mean age (years)	38.2 ± 12.4	15–63
Duration of dialysis (months)	36.8 ± 48.3	1–240
No. of HLA-compatible matches	2.0 ± 3	0–6
No. of HLA mismatches	3.5 ± 1.40	0–6
No. of units of blood	3.6 ± 2.8	1–21
No. of rejection episodes/patient	0.9 ± 0.8	0–3
Time of onset of rejections (days)		
First (n = 52)	53.4 ± 42.6	6–176
Second (n = 18)	103.8 ± 47.6	31–178
Third (n = 1)	105	

Table 2. Data concerning CMV infection and disease in the entire study population

	Values	Range
CMV-positive patients	38/78 (48.7%)	–
CMV-positive donors	35/72 (48.6%)	–
Mean time of onset of viruria (days)	68.8 ± 34	31–157
Mean time of onset of viremia (days)	55.2 ± 27.7	22–10
Infection incidence	35/78 (44.9%)	–
Disease incidence	14/78 (17.9%)	–
Disease severity		
Mild	5 (6.4%)	–
Moderate	6 (7.7%)	–
Severe	3 (3.8%)	–
Fatal	0	–

Results

General trends

Table 1 summarizes the general characteristics of the patients enrolled in the study. An analysis of data obtained from the 78 patients showed a global incidence of 44.9% for CMV infection and 17.9% for CMV disease during the first 6 months after transplantation (Table 2). There were five mild, six moderate, and three severe cases of CMV disease. There were no deaths attributable to CMV infection. Viremia occurred on the average 12–13 days before viruria (Table 2) and was present in all symptomatic patients regardless of prophylactic treatment used. The incidence of CMV infection and disease was higher in the subgroup at increased risk for CMV infection, i.e., seronegative recipients of seropositive donors (R– D+): 64.7% and 47.1%, respectively.

The first rejection episode preceded the occurrence of viremia by an average of 2–3 days. It was unclear whether this was due to a causal temporal effect or whether it was purely coincidental.

Effects of acyclovir

The acyclovir-treated group (group 3) and the controls (group 4) were comparable with respect to recipient and donor age, duration of dialysis, initial nephropathy, and number of blood transfusions (Table 3). There were fewer treated rejection episodes in the acyclovir group than in the control group (0.6 ± 0.7 vs 1.1 ± 0.8); $P < 0.05$). Subgroup distribution differed slightly between the two groups, with the acyclovir group containing more R– D+ recipient-donor pairs (patients at increased risk for primary infection) than the control group: 9/21 (42.9%) versus 3/32 (9.4%). This latter factor could have masked the protective role of acyclovir.

The rate of infection and disease in acyclovir-treated patients was 81.0% and 43.8%, respectively (Table 4). The probability of surviving infection was significantly higher in controls ($P = 0.002$, generalized Wilcoxon = 9.5). The rate of CMV disease, although higher in acyclovir-treated patients, was not statistically different from that of controls (38.1% vs 15.6%). Severity of disease was not different between groups, but of the eight symptomatic patients in the acyclovir group, two had severe disease with life-threatening interstitial pneumonia.

The time of onset of symptoms was delayed in the acyclovir group (56.7 days) compared to that in historical controls (29.2 days), but the difference did not reach statistical significance.

Acyclovir was well tolerated and no patient had to suspend treatment for drug toxicity. On the other hand, we did not observe any recurrent mucocutaneous herpes simplex infection in this treated group.

Seronegative blood products

Of the 19 seronegative patients who received kidneys from seronegative donors, only one patient developed CMV infection and disease. This patient had received two unscreened units of blood, and it is quite likely that the unscreened blood was the source of contamination. The use of seronegative blood products in this subgroup of patients proved to be highly effective in preventing primary CMV infection.

CMV-specific immunoglobulins

Three patients out of six in this group (50%) showed evidence of CMV infection, including one who developed mild disease. The small size of this group did not allow any statistical comparison to be made. Although the rate of infection did not seem to be reduced, the severity of disease tended to be lowered (one mild disease out of six: 16.7%), the overall disease incidence in R– D+ patients being 47.1% in this study population.

Discussion

This retrospective analysis showed an overall incidence of CMV infection and disease of 44.9% and 17.9%, respectively. These results are within the range of those reported in the literature. The different prophylactic strategies

Table 3. Distribution of factors influencing CMV infection in the acyclovir and control groups. * $P \leq 0.05$

	Acyclovir ($n = 21$)	Controls ($n = 32$)	P value
Patient age (years)	41.6 ± 11.6	36.8 ± 12.0	0.15
Donor age (years)	30.6 ± 12.6	29.2 ± 10.7	0.67
Duration of dialysis (months)	48.0 ± 65.4	31.9 ± 39.7	0.27
No. of HLA-compatible matches	2.3 ± 1.3	1.7 ± 1.4	0.12
No. of HLA mismatches	3.3 ± 1.2	3.8 ± 1.6	0.25
No. of units of blood	3.2 ± 2.0	4.0 ± 4.2	0.46
No. of rejection episodes	0.6 ± 0.7	1.1 ± 0.8	0.048*
Subgroup distribution			
D + R +	7	10	0.90
D + R −	9	3	0.01*
D − R +	5	13	0.30
D?		6	

Table 4. Comparison of CMV infection and disease between acyclovir and control groups. * $P \leq 0.05$

	Acyclovir ($n = 21$)	Controls ($n = 32$)	P value
Viruria			
– Incidence (%)	81.0	40.6	0.004*
– Time of onset (days)	67.9	69.0	0.93
Viremia			
– Incidence (%)	71.2	37.5	0.006*
– Time of onset (days)	56.0	56.3	0.98
Infection incidence (%)	81.0	43.8	0.007*
Disease incidence (%)	38.1	15.6	0.10
Time of onset of first symptom	56.7	29.2	0.128
Disease severity			0.29
– Mild	2	2	
– Moderate	4	2	
– Severe	2	1	

used in our center were influenced by results obtained from placebo-controlled, randomized trials and account for our varying options with time. We confirm the high efficacy of screened, CMV-negative blood products in preventing CMV infection in the subgroup of R– D– recipients. Indeed, only one patient out of 19 in this group presented with CMV symptomatic infection. In retrospect, it is likely that this patient was contaminated by unscreened blood products, although we cannot rule out false CMV-negative serology during screening in recipients or donors.

Six patients at increased risk for CMV primary infection received CMV Ig. The size of this subgroup of patients was too small to allow any statistical comparison to be made, but patients tended to have reduced disease incidence and severity, while the infection rate was 50% (3/6). It is interesting to note that in a study conducted by Snydman and colleagues [23], immunoglobulin preparations reduced the incidence of CMV disease from 60% to 21% in renal allografts. More recently, Plotkin and colleagues demonstrated that previous vaccination of seronegative renal transplant recipients with live Towne strain CMV reduces disease severity without inducing latency or increasing incidence of neoplasm [16]. The results of these studies and the fact that reinfection causes less severe disease than primary infection [9, 10] confirm the role of humoral immunity in controlling CMV infection [21].

We found considerably high infection and disease rates in patients prophylactically treated with acyclovir. In contrast to the results obtained by Balfour and colleagues [2], acyclovir did not reduce the rate of infection or disease in patients at risk for CMV infection when compared to patients without prophylaxis. It is possible that the significantly greater number of patients at increased risk for primary CMV infection (i.e., R– D+) in the acyclovir group accounted at least in part for this discrepancy in the present study. In addition, we cannot completely exclude other biases due to retrospective analysis. Although these factors could have masked a protective role of acyclovir, we observed unacceptably high rates of infection and disease in this treated group, and it is unlikely that methodological biases alone could have accounted for these unfavorable results. Patients at increased risk for primary CMV infection were not protected by acyclovir. Five out of nine R– D+ patients in this subgroup presented with CMV disease, including two with severe interstitial pneumonitis. This observation contrasts with the previous study conducted by Balfour and colleagues, who documented a greater protective effect of the same doses of acyclovir in this subgroup of patients.

Whether acyclovir can really prevent CMV infection and disease in every setting of renal transplantation remains questionable. This issue will have to be addressed in further randomized, controlled trials. In vitro anti-human CMV activity of acyclovir is rather weak. Only at concentrations greater than 100 μmol was a reduction in plaque formation of more than 50% observed in various clinical isolates [5, 13]. Peak plasma concentrations with oral acyclovir are well below these values [7]. This agent, an analogue of 2′-deoxyguanosine, needs to be phosphorylated by a viral thimidine kinase before it is converted to its triphosphate, a potent inhibitor of viral DNA polymerase. Unfortunately, CMV does not appear to code for a virus-specific thymidine kinase [6]. The hypothesis that human cells could monophosphorylate acyclovir [2] needs confirmation in further studies.

On the basis of these reported results, high-dose acyclovir for CMV prophylaxis was discontinued in our center. Seronegative patients with seronegative kidney donors (R– D–) continue to receive screened, CMV-negative blood products, and high-risk patients (R– D+) are treated with hyperimmune CMV Ig. As for the other subgroups of patients, i.e., R+ D+ and R+ D–, they rely on their pretransplant immunity to control, at least in part, CMV replication.

We conclude that given the observed high rate of infection in treated patients, high-dose acyclovir used for CMV prophylaxis does not reduce the infection rate in our renal transplant patients. These disappointing results call for

further clinical trials with newer antitiviral agents [20], alone or in combination with immunization modalities. The use of CMV-seronegative blood products should be recommended in R– D– patients.

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