Transpl Int (1994) 7 [Suppl 1]: S331–S335

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Cytomegalovirus (CMV) prophylaxis by acyclovir in pre-transplant CMV-positive renal transplant recipients

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Abstract Cytomegalovirus (CMV) infections, either primoinfection or reactivation, remain an important problem in organ transplantation. We therefore designed a prospective study in which pre-transplant CMV-positive renal transplant (RT) patients were randomized to receive for 3 months starting immediately after transplantation either acyclovir or nothing. Between April 1992 and January 1993, 53 cadaveric renal transplantations were performed in our institution. The immunosuppressive regimen included anti-thymoglobulins (ATG), azathioprine, steroids and cyclosporine A. Patients randomized in the acyclovir arm received the drug from day 1 to day 90 (D90) intravenously as long as the creatinine clearance was not above 10 ml/min and per os afterwards (3200 mg/day if the creatinine clearance was above 50 ml/min). CMV viraemia tests were systematically performed every 2 weeks until day 90 or when febrile episodes occurred. The patients were 53 adults who received a RT during the study period; 37 were included in the study of which 19 received acyclovir prophylaxis (group A) and 18, no prophylaxis (group B). The two groups did not significantly differ according to sex ratio, recipient's age, number of CMV-negative donors and number of days on ATG (10.76 ± 6.16 vs. 8.28 ± 4.21 days). There were significantly fewer viraemia episodes in group A ($n = 6$) than in group B ($n = 13$, $P < 0.05$); nevertheless, the percentage of symptomatic CMV viraemia was the same in both groups (35% vs. 38.5%). The onset of CMV viraemia occurred in the same period in both groups (39 ± 13.8 days vs. 34.3 ± 15 days; $P = \text{NS}$). The number of rejection episodes in the study period was the same in both groups (8 in each). We conclude from this prospective study that post-RT acyclovir prophylaxis reduces significantly the number of CMV viraemia episodes but does not delay their onset. Furthermore, it has no effect upon the percentage of symptomatic viraemias.

Key words Renal transplantation
Acyclovir · Cytomegalovirus
Immunosuppression

Introduction

Cytomegalovirus (CMV) has become the single most important pathogen after organ transplantation [10]. It is ubiquitous, with 50.80% of people developing CMV antibodies at some time during their life. CMV infections are usually asymptomatic in the general population but are potentially life-threatening in immunosuppressed transplant patients [13] and are associated with an increased number of acute organ rejections [7]. The three potential sources of CMV infection in the transplant setting are (1) the donor organ, (2) cellular blood products and (3) the reactivation of endogenous virus [1, 4, 14].

Among the methods of preventing CMV infections, the use of (1) CMV-seronegative blood products, (2) intravenous high titer CMV hyperimmune globulins and (3) prophylaxis with antiviral agents such as acyclovir and ganciclovir has been demonstrated to be efficient [2, 8, 11]. Thus, Balfour et al. [2] demonstrated in a prophylactic randomized study testing high-dose oral acyclovir versus placebo that the incidence of CMV infections was reduced from 61% to 36%; nevertheless, the incidence of CMV disease was significantly decreased only in the group donor CMV+/recipient CMV−. Based on this study, two recent papers showed controversial results. Legendre et al. [6] found in a prospective non-randomized study that high doses of acyclovir significantly decreased both CMV infection and CMV disease in the CMV+ recipient group independently of the donor CMV serology. In contrast, Wong et al. in a retrospective study did not find a significant decrease of CMV infection or CMV disease when the recipients were given acyclovir 3200 mg/day for 3 months [15]. On the basis of these results, we conducted a prospective randomized study in which we offered to CMV-positive renal transplant patients either prophylactic high-dose acyclovir or nothing.

Materials and methods

Patients

Between April 1992 and February 1993, 53 cadaveric renal transplantations were performed in our institution. Of these patients 37 (i.e. 70%) were CMV-seropositive before transplantation. All the patients received the same sequential immunosuppression, i.e. antithymoglobulins (ATG) until the serum creatinine was below 200 μmol/l, then cyclosporin A (CsA) 6 mg/kg daily adapted to blood trough levels (120–150 ng/ml); azathioprine (AZA) and prednisolone (CS) were started immediately before transplantation. The AZA dosage (2 mg/kg daily) was adapted to the number of platelets ($\geq 150000/mm^3$) and white blood cells ($\geq 3000/mm^3$). CS was initially given at 1 mg/kg daily for 2 weeks and then progressively tapered to 10 mg/day by the end of the 3 post-transplant month.

Rejections were diagnosed on clinical and histological grounds; they were treated by methylprednisolone pulses (5 mg/kg daily for 3 days). If no improvement was noted OKT3 was given for 10 days (5 mg/day). We analysed the patients who had been followed up post-transplant for at least 2 months.

CMV serological status

The CMV serological status was determined just prior to organ procurement in the donor and before transplantation in the recipient by the detection of CMV-specific immunoglobulin (Ig)G by an indirect immunofluorescent assay.

Virus cultures

Blood specimens were collected in heparinized vials. After decantation CMV detection was performed by two methods: (1) conventional culture carried out by inoculation of MRC5; (2) rapid diagnosis. In this latter technique, 100 μl of buffy coat were inoculated in duplicate in 24-well dishes seeded with MRC5. Dishes were centrifuged at 3500 rpm for 1 h and inoculated overnight at 37 °C in a 5% CO_2 atmosphere. After acetone/water fixation, CMV immediate early antigen (IEA) was detected in the MRC5 nucleus by an immunoenzymatic reaction using an anti-IEA monoclonal antibody (E13 Biosoft).

Definition of CMV infection versus disease

CMV infection was defined as the isolation of CMV from blood in the absence of clinical symptoms such as fever arthralgias, dyspnoea, gastrointestinal symptoms or clinical findings (leukopaenia, thrombopaenia, pulmonary infiltrates, hepatitis, retinitis, enteritis). *CMV disease* required laboratory documentation of CMV infection; in addition, the patient had to be febrile (with a rectal temperature of 38.5°C for at least 2 days within a 7-day period) with or without respiratory, renal, hepatic, haematologic, gastrointestinal or musculoskeletal findings that could not be attributed to another pathogen.

Regimen of prophylaxis

All CMV-positive adult recipients of a cadaveric transplant were eligible for this study, which began in April 1992. The patients were randomized to receive either acyclovir or nothing for the first 3 post-transplant months. None of the patients received anti-CMV immunoglobulins. Intravenous acyclovir was begun immediately after transplantation, i.e. 6 mg/kg the first day. The intravenous route was used for the first 3 post-operative days and/or until creatinine was above 10 ml/min (i.e. 6 mg/kg daily). Afterwards, the oral route could be used with dosage adjustment to creatinine clearance as given in Table 1.

The patients were evaluated daily after surgery until discharge. CMV evaluation, i.e. systematic blood and urine cultures, were performed every 2 weeks until day 90, when the patients were seen in the outpatient clinic, and during any postoperative in patient admissions. Any sign or symptoms suggestive of CMV infection disease was recorded.

Table 1 Adjustment of dosage after day 3 postoperatively

Creatinine clearance (ml/min)	IV dosage (per day)	Oral dosage (per day)
≦ 10	6 mg/kg	–
< 10 ≦ 25	12 mg/kg	800 mg × 3
< 25 ≦ 50	12 mg/kg × 2	800 mg × 4
> 50	12 mg/kg × 3	800 mg × 4

Study endpoints

When a patient presented with either asymptomatic viraemia or asymptomatic viruria, acyclovir was not discontinued (in the acyclovir arm), and in both groups the patients did not receive additional treatment, i.e. ganciclovir or foscarnet. When CMV infection or CMV disease occurred, the study was stopped, and the patients were offered supportive care, i.e. ganciclovir 10 mg/kg daily for 15 days. The rates of CMV disease and the survival of both graft and patients were considered the endpoints of the study.

Statistical analysis

Data are expressed as mean ± SD. Univariate analysis was performed by Student's *t*-test for continuous variables and by χ^2 test for categorical variables.

Results

The acyclovir-treated group ($n = 19$, group A) and the controls ($n = 18$, group B) were comparable according to sex ratio, recipient's age, time on dialysis and donor CMV serology (Table 2). The analysis of data obtained from the 37 patients (Table 3) showed a significant decrease of CMV infection in group A, whereas the incidence of CMV disease was similar in both groups, i.e. low (nevertheless, CMV disease was more severe in group B). Thus, we only observed one case of CMV disease in group A (fever, leukopaenia, thrombopaenia) and two in group B (fever, dyspnoea, lung infiltrates, CMV in the bronchoalveolar lavage). The patients responded to ganciclovir; we did not observe any CMV-related death. The time to onset of CMV infection/disease was not statistically delayed in the acyclovir group (41.3 days) compared with the control group (38.3 days). Acyclovir was well tolerated; we did not observe any drug-related toxicity. The compliance was good, i.e. no patient discontinued the treatment. Finally, we did not observe recurrent mucocutaneous herpes simplex infections in the treated group while the patients were receiving acyclovir. Finally, the patient and graft survival rates were similar in both groups, with a mean follow-up of 11.8 months in group A and 12.1 months in group B.

Discussion

CMV infection and CMV disease remain the most important infectious problems after organ transplan-

Table 2 Characteristics of the study population (*ATG* anti-thymoglobulins, *CMV* cytomegalovirus, *RT* renal transplant)

	Group A (acyclovir) $n = 19$	Group B (no prophylaxis) $n = 18$	*P*
Sex ratio (M/F)	13/6	14/4	NS
Recipient's age (years)	50.4 ± 11.3	45.1 ± 11.1	NS
Time on dialysis (months)	59.75 ± 69.2	59.2 ± 47.2	NS
Number of transplantations	1.19 ± 0.5	1.25 ± 0.5	NS
Days of ATG	10.76 ± 6.16	8.28 ± 4.21	NS
Vials of ATG/patient	22.7 ± 12.4	19 ± 7.8	NS
Donors CMV +	9/19	8/18	NS
Donors CMV −	10/19	10/18	NS
Mean follow-up since RT (months)	11.8 ± 3.5	12.1 ± 4.7	NS

Table 3 Prevalence of CMV infection/disease

	Group A ($n = 19$)	Group B ($n = 18$)	*P*
CMV infection	5	11	< 0.05
CMV disease	1 [a]	2 [b]	NS
Onset of CMV infection/disease (day since RT)	41.3 ± 15	38.3 ± 15.4	NS
Number of rejection episodes	8	8	NS
Number of patients with rejections	7	8	NS
Patient survival	100%	94.4%	NS
Graft survival	89.5%	94.4%	NS

[a] Fever, leucopaenia, thrombopaenia
[b] Fever, cough, CMV in bronchoalveolar lavage in both cases

tation, usually occurring in the first 3 months following transplantation [10]. Ho et al. [5] and Betts et al. [3] were the first to provide epidemiological evidence for the transmission of CMV via the transplanted kidney. CMV may also contribute to other morbidities, including an increased susceptibility to other infectious agents [9] and an increased risk of rejection and graft dysfunction [7]. To date, no efficient anti-CMV vaccine is available to protect potential organ recipients who are CMV-seronegative [12]. Balfour et al. [2] were the first to demonstrate that high-dose acyclovir (i.e. 3200 mg/day) for the first 3 post-transplant months was able to protect RT patients from CMV infection, especially in the high-risk group, i.e. donor CMV-seropositive/recipient CMV-seronegative. More recently, two controversial non-randomized studies have been reported on the use of acyclovir to prevent CMV infection/disease. The first one [6] demonstrated a significant decrease of both CMV infection and CMV disease with acyclovir in the CMV-seropositive recipient group. In contrast, Wong et al. [15] failed to demonstrate a clear benefit of acyclovir therapy in RT patients if their pre-transplant serological status was negative. To date, our study is the first prospective randomized one to test the efficacy of acyclovir at preventing CMV infection disease in CMV-seropositive RT patients. High doses of acyclovir (i.e. 3200 mg/day) adjusted to creatinine clearance are well-tolerated since we did not observe adverse effects possibly related to the drug. Moreover, we did not observe herpes simplex virus type 1 or 2 infections when the patients were receiving acyclovir. The percentage of overall CMV infection disease is significantly less in the acyclovir group; in fact, the number of CMV infections (i.e. asymptomatic viraemias) is less, whereas the incidence of CMV disease is similar in both groups; nevertheless, CMV diseases were more severe in the group without acyclovir, i.e. the patients were febrile and dyspnoeic and had CMV in their bronchoalveolar lavage. One patient died in group B, but his death was not related to CMV disease since he presented 3 weeks after CMV disease with a *Legionella*-related pneumonia associated with a fatal haemophagocytic syndrome. The onset of CMV infection/disease was not significantly delayed in the acyclovir group (41.3 ± 15 days versus 38.5 ± 15.4 days in group B).

The number of rejections was similar in each group (43%), their onset was not significantly delayed in the acyclovir group, and we did not observe a correlation between the onset of CMV viraemia and the development of rejection in the preceding or following days (data not shown), but the number of patients is too small to draw any firm conclusion.

With a mean follow-up of 1 year, the patient and graft survival rates are not statistically different between the groups. Thus, we wonder whether the decrease of CMV infections has an impact on the long-term graft outcome. On the contrary, the decrease of CMV disease is an important goal to achieve, but our study is too small to answer this question. Therefore, at least 40 more patients will have to be included in this prospective study if we want to demonstrate a clear benefit of high doses of acyclovir in CMV-seropositive renal transplant patients.

In conclusion, this prospective study, although not complete, does demonstrate that high doses of acyclovir (i.e. 3200 mg/day) given during the first 3 post-transplant months to renal transplant patients are able to significantly decrease the incidence of CMV infection; nevertheless, the incidence of CMV disease was not affected. The number of rejection episodes was similar in both groups. Thus, the use of high doses of acyclovir in these patients is questionable.

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