Impact of High-Dose Oral Acyclovir Prophylaxis on Cytomegalovirus (CMV) Disease in CMV High-Risk Renal Transplant Recipients

Josef Kletzmayr, Harald Kotzmann, Theresia Popow-Kraupp, Josef Kovarik, and Renate Klauser

J. Kletzmayr, H. Kotzmann, J. Kovarik, R. Klauser, Clinic for Internal Medicine III, Department of Nephrology and Dialysis, University of Vienna, Vienna, Austria
T. Popow-Kraupp, Institute of Virology, University of Vienna, Vienna, Austria

(Received May 12, 1995. Accepted August 16, 1995.)

Cytomegalovirus (CMV) infection is the most important infectious complication in immunosuppressed transplant recipients. The incidence of CMV infection varies between different transplant populations; in renal transplant recipients it has been reported to be as high as 48 to 100% (1,2) before the introduction of cyclosporine A (CsA) and similar values (37 to 72%) were reported in kidney allograft recipients treated with CsA and prednisolone (2-4). The additional use of mono- or polyclonal antibodies given either prophylactically or for rejection therapy increases the risk of viral infection (3-6).

Symptomatic CMV disease as a major cause of morbidity and mortality in renal transplant recipients develops more often in primary infections (recipient CMV seronegative, R-) carrying a probability of disease of 50 to 60% compared with 20% in secondary infections (recipient CMV seropositive, R+) (1,2,6). Because the kidney of a seropositive donor is a frequent source of infection, an important risk factor for CMV disease is seropositivity of the donor (D+) and seronegativity of the recipient (R-) with a majority of studies showing a significantly higher incidence of infections compared with D-R- and more severe courses of disease compared with other seroconstellations (2,3,7-10).

Contradictory results have been reported concerning the prophylactic efficacy of oral acyclovir, which is a well-established agent in the treatment of herpes virus infections (11). In 1989, Balfour et al. (12) reported high-dose oral acyclovir prophylaxis to be very effective in all renal transplant recipients at risk. Especially in D+R- patients, the incidence of disease could be reduced significantly. These results could not be confirmed by other investigators (13-16).

Stimulated by the promising results reported by Balfour et al. (12), we aimed to reevaluate oral acyclovir prophylaxis in a larger number of high-risk (D+R-) kidney recipients who are expected to develop the more severe course of CMV disease and therefore could benefit most from an effective prophylactic regimen.

PATIENTS AND METHODS

We included 36 seronegative recipients who were receiving renal allografts from seropositive donors in this open-labeled, randomized controlled study. The study protocol was approved by the local ethical committee and written informed consent was obtained before study entry. Patients were randomly assigned to prophylactic treatment with oral acyclovir (ACY, N = 24) or to the control group (CO, N = 12), which received no CMV prophylaxis. Patients were randomized to ACY:CO in a 2:1 ratio because a highly beneficial
effect of prophylaxis was expected. Oral acyclovir prophylaxis in the treatment group (Zovirax®; Wellcome, London, England) was administered in the same dosage as originally used by Balfour et al. (12): patients received 1 × 800 mg on the day of transplantation, 2 × 800 mg on the first postoperative day and then, according to renal function, up to 4 × 800 mg/day for 3 months after TX or until diagnosis of CMV disease necessitating treatment. The final number of patients evaluated was 32. One patient was excluded from final analysis because acyclovir prophylaxis was administered less than 1 month after TX and one patient was excluded because his serostatus before transplantation could not be confirmed, as seroconversion occurred within 1 wk after TX. Two patients were excluded because of loss to follow up.

CMV immunoglobulin G (IgG) antibodies of donors and recipients were determined by an ELISA (17) before TX. CMV serostatus of recipients was determined additionally by a passive latex agglutination test (CMV Scan®; Becton Dickinson, Cockeysville, MD) (18) before TX with the results available within 30 min. Recipients were considered to be CMV seronegative when both tests were negative in accordance with the patient’s serostatus at the time of evaluation for renal TX.

The two groups included in the final analysis were comparable with regard to age, sex, HLA mismatches (maximal sum of mismatches accepted according to Eurotransplant guidelines: HLA-B,DR = 2), polyclonal antibodies (PRA) before TX, HLA-DRw6-positivity (19), and baseline immunosuppression (IS) (Table 1). Baseline immunosuppressive therapy consisted of CsA (Sandimmun®; Sandoz, Basel, Switzerland) (5 mg/kg iv for 3 to 4 days, then orally daily, adjusted to trough level) and methylprednisolone iv for 5 to 6 days followed by 20 mg oral prednisolone, with dose reduction after 3 months. Patients with 5 to 40% PRA before transplantation received triple therapy with CsA, prednisolone, and azathioprine (100 mg/day initially, adjusted to leukocyte count); patients with PRA > 40% were given quadruple therapy with an additional 10-day course of induction therapy with antithymocyte globulin (2.5 mg/kg per day) (ATG®, Fresenius, Bad Homburg, Germany). Biopsy-proven acute rejection episodes were treated with 500 mg methylprednisolone for 3 days and thereafter, in case of persistence, according to renal function, up to 4 × 10-day course of induction therapy with CsA, prednisolone, and azathioprine: quadruple therapy: CsA, prednisolone, azathioprine plus ATG-induction therapy.

Serological diagnosis of CMV infection was made on the basis of measurement of CMV-specific IgG- and immunoglobulin M (IgM)-class antibodies by ELISA, by using standard techniques (17) with a special antigen preparation (20). Urinary CMV excretion was determined by means of virus isolation and monoclonal antibodies against the early nuclear antigen (21,22). CMV antigen in peripheral blood leukocytes was detected by monoclonal antibodies against P (21,22). Screening for CMV infection was performed weekly during the initial hospitalization, bieweekly for 3 months and thereafter monthly for 1 yr. Transplant nephrectomy patients were screened for at least 1 additional month. The onset of CMV infection was defined as the day of first confirmation by one of the diagnostic methods cited above. CMV disease was defined as the occurrence of at least one CMV-related symptom (fever, leukocytopenia, thrombocytopenia, pneumonia, hepatitis, reinitis, gastroenteritis, graft dysfunction, involvement of central nervous system, opportunistic infections) with confirmation of CMV infection. Severity of disease was quantified by a scoring system described by Smiley et al. (9) and modified as outlined in Table 2. Individual severity score was defined as the sum of maximum scores in each category. Symptoms were not scored when attributable to other causes. CMV disease was treated with ganciclovir (Cymevene®, Grunenthal, Stolberg, Germany) 5 mg/kg given

### TABLE 1. Patient characteristicsa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ACY (N = 22)</th>
<th>CO (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 ± 14</td>
<td>44 ± 13</td>
</tr>
<tr>
<td></td>
<td>(range, 26 to 71) (range, 20 to 61)</td>
<td></td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>5/17</td>
<td>3/7</td>
</tr>
<tr>
<td>PRA (%)</td>
<td>7 ± 11</td>
<td>5 ± 13</td>
</tr>
<tr>
<td></td>
<td>(range, 0 to 43) (range, 0 to 51)</td>
<td></td>
</tr>
<tr>
<td>HLA-DRw6+ (%)</td>
<td>8 (36.4)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Mismatch HLA-B,DR</td>
<td>1.3 ± 0.9</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>Baseline IS: N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsA, prednisolone</td>
<td>15 (68.2)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Triple therapy</td>
<td>6 (27.3)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Quadruple therapy</td>
<td>1 (4.5)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

a Triple therapy: CsA, prednisolone, azathioprine; quadruple therapy: triple therapy plus ATG-induction therapy.

b PRA, polyclonal antibodies.

c HLA-DRw6+ , HLA-DRw6 positivity.

### TABLE 2. Scoring system for CMV diseasea

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Severity</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever &gt;37.5°C</td>
<td>&lt;39.0°C</td>
<td>2</td>
</tr>
<tr>
<td>Leukocytopenia*</td>
<td>Decrease &gt;4.0 g/L</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&lt;4.0 g/L</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt;2.0 g/L</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&lt;1.0 g/L</td>
<td>6</td>
</tr>
<tr>
<td>Thrombocytopenia*</td>
<td>Decrease &gt;100 g/L</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&lt;100 g/L</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt;60 g/L</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&lt;40 g/L</td>
<td>6</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Typical chest X-ray</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>+ Dyspnea</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>+ Respirator</td>
<td>9</td>
</tr>
<tr>
<td>Opportunistic Infections</td>
<td>Reversible</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Irreversible</td>
<td>6</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>Diarrhea</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>GI bleeding</td>
<td>9</td>
</tr>
<tr>
<td>Graft Dysfunction</td>
<td>Serum creatinine</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>&lt;177 μmol/L</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;354 μmol/L or return to HD</td>
<td>9</td>
</tr>
<tr>
<td>CNS Involvement</td>
<td>Lethargy</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Stupor</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Coma</td>
<td>9</td>
</tr>
<tr>
<td>Hepatitis (ALT)</td>
<td>&gt;50 U/L</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>&gt;200 U/L</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;500 U/L</td>
<td>9</td>
</tr>
</tbody>
</table>

a Leukocytopenia and thrombocytopenia: a significant decrease of leuko- or thrombocytes was considered as CMV-related symptom; GI, gastrointestinal; HD, hemodialysis; CNS, central nervous system; ALT, alanine amino transferase.
iv twice a day with dose adjustment according to transplant function for 10 to 14 days or in combination with a therapeutic dose of CMV hyperimmune-globulin (Cytotoxic®, Biotest, Dreieich, Germany).

Statistical evaluation of the data was done by using t test, Fischer’s exact test, and Mann-Whitney U test as appropriate. A difference was considered statistically significant when the P value was < 0.05. Results are expressed as mean ± SD.

RESULTS

Prophylaxis

CMV infection was confirmed in 68.2% of patients in the acyclovir-treated group (ACY, N = 15 of 22) and in 50% in controls (CO, N = 5 of 10) (not significant [NS]). CMV disease occurred in 40.1% (ACY, N = 9 of 22) and 40% (CO, N = 4 of 10), respectively (NS). Thus, the incidence of disease in patients with confirmed CMV infection was slightly lower in the treatment group compared with controls (ACY, 60%; CO, 80%; N = 9 of 15 versus CO, 80%; N = 4 of 5; NS). Neithervery (ACY, 11.4 ± 5.5 versus CO, 12.5 ± 7.9 points maximal score; NS) nor duration of disease (ACY, 20.9 ± 8.8 versus CO, 21.8 ± 11.4 days; NS) differed significantly between the two groups. All but one patient with CMV disease in each group developed fever. Leukocytopenia and thrombocytopenia (for definitions, see Table 2) occurred in the majority of patients in both groups (leukocytopenia: ACY, 66.7%; CO, 50%; thrombocytopenia: ACY, 55.6%; CO: 75%).

A temporary elevation of serum creatinine levels not attributable to rejection episodes was observed in 66.6% (ACY) and 100% (CO), respectively. CMV-related pneumonia was clinically diagnosed in one patient in each group. No cases of CMV retinitis, gastroenteritis or central nervous system symptoms occurred. None of the patients in either group died and no relevant side effects of acyclovir prophylaxis occurred. The onset of CMV disease was not significantly delayed in the acyclovir group (47.3 ± 33.8 days after TX) compared with the control group (27.2 ± 14.4 days) (P = 0.454, NS) (Figure 1). Development of CMV infection and disease were neither associated with HLA-DRe6 positivity (15) nor with the number of packed red-cell concentrates (leukocyte depleted, irradiated, CMV negative) administered in the postoperative period (data not shown).

Therapy

Treatment of CMV disease with ganciclovir was given over a period of 11 days (10 ± 2 days). Five patients received ganciclovir in combination with CMV hyperimmune globulin. One patient in the acyclovir group recovered from CMV disease without any therapy as an outpatient. The recurrence rate of CMV disease requiring a second course of treatment with ganciclovir (and CMV-hyperimmune globulin) was similar in both groups (ACY, 33.3% versus CO, 25%, of cases with disease; NS).

Rejection Episodes

The number of patients receiving a steroid pulse because of deterioration in creatinine measurement (ACY, 90.1% versus CO, 80%; NS), the percentage of biopsy-proven rejections (ACY, 59.1% versus CO, 40%; NS) and the therapeutic use of mono- or polyclonal antibodies (ACY, 54.5% versus CO, 30%; NS) did not differ significantly between the two groups. There was a trend toward a higher incidence of CMV disease after prophylactic or therapeutic administration of mono- or polyclonal antibodies (47%) compared with patients not treated with antibodies (33%; NS).

Graft function after 1 yr, as assessed by serum creatinine level and number of graft failures (N = 4), was similar between the acyclovir and the control group, and better in both groups without CMV disease (data not shown). All cases of graft failure were the result of rejections: two transplant nephrectomies in the acyclovir group were caused by parenchymal necrosis because of vascular rejection and were performed 68 and 43 days after TX. One transplant nephrectomy in the control group was done because of acute vascular rejection, and one patient returning to hemodialysis had a history of biopsy-proven chronic vascular rejection. All patients with graft failure had a history of CMV disease and two out of three patients with recurrent CMV disease in the acyclovir group lost graft function within 1 yr after TX. No interstitial CMV infection of the allograft was detected in any of the biopsies.

DISCUSSION

Different prophylactic regimens—active immunization (25), passive immunization (26–29), interferon (30), CMV matching (31–33), prophylactic (34,35) and preemptive (6,29,34,36) administration of ganciclovir, and combinations of antiviral agents (34,37)—have been evaluated with respect to their potency to reduce CMV-related complications in renal transplant recipients. None of these strategies proved entirely satisfactory in the management of patients at risk (donor and/or recipient CMV-seropositive) of CMV disease.
and, consequently, there are no clear recommendations for the prevention of CMV infection and disease.

Although specific therapy with ganciclovir is now available, prevention of CMV infection and disease still has an important bearing on the outcome of renal transplantation. Besides CMV-related morbidity, overt CMV disease might influence graft and patients' survival rates (2,7-3,38). However, this is not a universal finding and the relationship between CMV disease and rejection or acute transplant glomerulopathy remains controversial (38-43).

In 1989 Balfour et al. (12) reported that high-dose oral acyclovir prophylaxis was highly effective in renal transplant recipients in reducing the incidence of CMV infection (virus isolation) and disease. This was a randomized, placebo-controlled double-blind study in all patients at risk, e.g., recipient and/or donor seropositive. The effect was greatest in the D+R- group, reducing the incidence of CMV-disease from 100% in the placebo group to 17% in the acyclovir group. Severity of CMV disease was not affected by acyclovir prophylaxis. These results were confirmed in an open-labeled uncontrolled study by Vasquez et al. (44), who reported an incidence of disease of 7% in 14 high-risk patients with acyclovir prophylaxis. Oral acyclovir has important advantages compared to other prophylactic regimens, including lower cost, widespread applicability to patients in different serostatations, and availability of oral therapy for use in outpatients.

Because of these promising results, we reevaluated the prophylactic benefit of oral acyclovir in high-risk renal transplant recipients in a relatively large randomized study. Our results show similar incidence of infection and disease and similar severity and duration of disease in the acyclovir-treated and control groups. Therefore, we could not confirm a beneficial effect of oral acyclovir prophylaxis. Compared with the control group, the rate of infection was slightly higher, and the proportion of CMV infections resulting in clinical disease was slightly lower in the acyclovir group. This difference could be the result of the higher incidence of rejection in the acyclovir group, requiring use of mono- and/or polyclonal antibodies as antirejection therapy. In contrast to other studies, we found a nonsignificant trend only toward higher incidence of CMV disease after administration of mono- or polyclonal antibodies (3-5). However, the influence of immunosuppression, especially of monoclonal antibodies, might be less pronounced in patients at risk of primary infections (6). The absence of severe CMV disease in our study may be attributable to the early start of therapy at the first sign of active CMV infection.

Our results concerning the delayed onset of CMV disease in the acyclovir group are in accordance with the findings of other investigators (13,14). It has been suggested that this delay itself could have a beneficial effect, but we did not find an effect of later onset of CMV disease on the severity of disease or transplant function. Seven out of nine patients in the acyclovir group developed CMV disease before the end of the 3-month course of acyclovir. HLA-DRw6 positivity of the recipient has been described as a risk factor for the development of active CMV infection (19), but in our study, HLA-DRw6 positivity was not associated with infection or disease.

Our data are in contrast to the promising results of Balfour et al. (12). The positive effect demonstrated by Balfour et al. in high-risk patients could have been overestimated because of the very high incidence of CMV disease (100%) in the placebo group of this study and the small number of patients involved (N = 13). On the other hand, the only study confirming these results (44) found a very low incidence of CMV infection (7% virus isolation or seroconversion) in high-risk patients treated with acyclovir.

In addition, pharmacological and pharmacokinetic data on acyclovir raise further questions about the use of this drug as a prophylactic agent. The antiviral pharmacological effect of acyclovir is dependent on the phosphorylation of acyclovir by a viral nucleoside kinase to the active viral DNA polymerase inhibitor. Because human CMV does not exhibit such a specific nucleoside kinase, acyclovir concentrations for 50% inhibition are high compared with those of ganciclovir (45). In spite of the clinically positive effect, the in vitro plasma acyclovir concentrations achieved with the dosing scheme of Balfour et al. (12) did not reach the levels for 50% inhibition in in vitro cultures (46). Although these data do not necessarily reflect the intracellular concentration of the active metabolite, they support our finding of the lack of prophylactic potency of acyclovir.

Our results are in accordance with the findings of Legendre et al. (15), who could not confirm a beneficial effect of high-dose oral acyclovir in a smaller group of high-risk patients (D+R-, N = 10) compared with historic controls. They found a significant reduction in the incidence of CMV infection and disease in CMV-seropositive renal transplant recipients receiving acyclovir prophylaxis (N = 32). Only in seropositive recipients was CMV infection delayed. Mortality did not differ between the two groups; severity of disease was not reported. Chitwood et al. (13) found a statistically insignificant reduction of the incidence of CMV disease in high-risk patients (D+R-, N = 28) with acyclovir prophylaxis compared with historic controls. Severity of disease was similar between the two groups. Bailey et al. (16) reported no change in the incidence of CMV disease after initiation of acyclovir prophylaxis (with or without intravenous immunoglobulin) in a small number of high-risk patients (renal allograft recipients, N = 14; acyclovir prophylaxis, N = 8) in their institution. In a retrospective analysis, Wong et al. (14) reported a higher incidence of CMV infection and disease in patients at risk (donor and/or recipient CMV seropositive) receiving oral acyclovir prophylaxis with one initial iv dose before surgery (N = 21), compared with historic controls receiving no prophylactic treatment (N = 32). This effect was
possibly attributable to a higher proportion of high-risk patients in the acyclovir group. Five out of nine D+R− patients in the acyclovir group developed CMV disease.

The immunosuppressive regimens used in the different studies of acyclovir prophylaxis cited above varied considerably. In the study by Balfour et al. (12), all patients received induction therapy with mono- or polyclonal antibodies and triple baseline immunosuppression (CsA, prednisolone, and azathioprine). In comparison, our immunosuppressive regimen was less aggressive, and this might account for the difference in prophylactic efficacy of oral acyclovir. Other investigators that used an immunosuppressive regimen similar to that of Balfour et al. (13–16) could not confirm a prophylactic benefit either. However, the influence of immunosuppression on CMV D+R− transplant recipients warrants further investigation (6).

We conclude that the prophylactic use of this type of nucleoside analog should be reconsidered. We are currently taking part in a double-blind randomized multicenter trial to evaluate the prophylactic potency of valacyclovir, the L-valyl ester of acyclovir (47), in renal transplant recipients. Because the bioavailability of acyclovir is reportedly higher for valacyclovir, the results of this large trial, which includes CMV-seropositive transplant recipients, should clarify the prophylactic benefit of acyclovir and its prodrugs in renal transplant recipients.

REFERENCES