Foscarnet for pre-emptive therapy of CMV infection detected by a leukocyte-based nested PCR in allogeneic bone marrow transplant patients

P Ljungman¹, G Öberg², J Aschan¹, A Ehrnström³, B Lönnqvist¹, K Pauksen² and P Sulila⁴

¹Department of Hematology, Institution of Internal Medicine, and ²Division of Clinical Virology, Huddinge University Hospital, Karolinska Institute, Huddinge; ³Department of Medicine, Uppsala University Hospital, Uppsala; and ⁴Astra Arcus AB, Södertälje, Sweden

Summary:

Fifteen allogeneic BMT patients in a phase II study were given foscarnet 60 mg/kg twice daily for 14 days as pre-emptive therapy against CMV disease. CMV infection was diagnosed by a leukocyte-based nested PCR. All 15 patients were evaluable for toxicity. One patient did not fulfill the inclusion criteria of two consecutively positive CMV PCR tests and therefore was not evaluable for efficacy. Thus, 14 of 15 patients were evaluable for development of CMV disease. None of the patients developed CMV disease and all 14 assessable patients had a negative CMV isolation at the end of therapy. None of the 15 patients had to discontinue therapy due to toxicity. Six patients reported mild gastrointestinal disturbances, three patients headaches, and three patients mild urethritis or hemorrhagic cystitis. Serum-electrolyte disturbances were common including abnormal magnesium, potassium and calcium levels. Two patients developed mild serum-creatinine increases requiring adjustment of the foscarnet dosage according to protocol. We conclude that a dosage of foscarnet of 60 mg/kg given twice daily seems to be safe and effective in preventing CMV disease in allogeneic BMT recipients. A study comparing foscarnet and ganciclovir is indicated.

Keywords: CMV; foscarnet; pre-emptive therapy; PCR

CMV infections have been a major cause of morbidity and mortality in allogeneic BMT patients. Recent advances in early detection and the use of antiviral chemotherapy have reduced the mortality in CMV disease. One of these important advances in CMV management is the use of pre-emptive therapy based on either detection of CMV antigenemia or PCR.¹⁻³ Two recent studies showed reductions of the risk for development of CMV disease in patients repeatedly tested by leukocyte-based PCR for CMV DNA compared with patients tested by rapid isolation techniques.⁴⁻⁵ Two antiviral drugs, ganciclovir and foscarnet, are currently widely available for use as pre-emptive therapy. The use of ganciclovir is hampered by bone marrow toxicity and the use of foscarnet by renal toxicity and the development of serum-electrolyte disturbances.²⁻⁵ The toxicity of foscarnet is dosage-dependent and the aim of this pilot study was to investigate the efficacy and safety of a lower dosage of foscarnet (60 mg/kg twice daily) as pre-emptive therapy in allogeneic BMT patients.

Patients and methods

Study design

The study design was an open one arm pilot study in which patients with CMV infection defined as two consecutive samples, collected within 7 days, positive for CMV DNA in leukocytes by a nested PCR were enrolled. Patients with verified or strongly suspected CMV disease were excluded from the study. The study period included 2 weeks of foscarnet therapy and 2 weeks of follow-up.

Patients

Fifteen allogeneic BMT patients were included in the study (13 from Huddinge and two from Uppsala). Patient characteristics at enrollment are shown in Table 1. The BMT procedure was published previously.⁶⁻⁷

<table>
<thead>
<tr>
<th>Table 1 Patients characteristics</th>
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<tbody>
<tr>
<td>Age (median; range) (years)</td>
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<tr>
<td>Time from BMT (days)</td>
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<tr>
<td>Diagnosis</td>
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<tr>
<td>Acute leukemia</td>
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<tr>
<td>Chronic myeloid leukemia</td>
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<tr>
<td>Severe aplastic anemia</td>
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<tr>
<td>Conditioning regimen</td>
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<tr>
<td>Cyclophosphamide + TBI</td>
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<tr>
<td>Cyclophosphamide + TLI + ATG</td>
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<tr>
<td>Cyclophosphamide + TBI + ATG</td>
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<tr>
<td>Acute graft-versus-host disease prophylaxis</td>
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<tr>
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<td>Grade II-IV</td>
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Correspondence: Dr P Ljungman, Dept of Hematology, Huddinge University Hospital, S-14186 Huddinge, Sweden
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Foscarnet therapy

All patients received foscarnet at a dosage of 60 mg/kg as 1-h infusions twice daily for 14 days with hydration with 500 ml of normal saline or 5% dextrose given together with all infusions. Patients regarded to be dehydrated received additional substitution to become normovolemic. The foscarnet dosage was adjusted according to estimated creatinine clearance. The foscarnet therapy was stopped after 14 days in all patients and no maintenance treatment was given.

Other therapy

No other antiviral therapy was allowed concurrently with foscarnet therapy. However, high dosage acyclovir given prophylactically was allowed before study entry but had to be stopped at study entry. High dosage i.v. standard immune globulin given once weekly as GYHD prophylaxis was permitted. It was recommended that other nephrotoxic agents such as amphotericin B and vancomycin should be avoided if possible.

Virology

Patients were tested weekly from transplant to study entry with a leukocyte-based nested PCR for CMV DNA performed in single tubes. The PCR technique is described below. When a patient tested positive by PCR a second sample of EDTA blood was drawn and analyzed for CMV DNA by PCR. A patient was eligible for the study if the second PCR was also positive. In addition determination of CMV early antigen by a rapid isolation culture technique and virus isolation was performed according to previously described techniques. The PCR technique has been described previously. Two x 10⁶ leukocytes were lysed in 50 μl of a buffer containing NP40, Tween 20 and proteinase K, and the lysate was thereafter inactivated at 95°C. Five microliters of the lysate were processed by a nested PCR in a volume of 50 μl. The primers used were from the conserved region of the CMV major immediate-early (IE) gene. The outer primers IEA-2A and IEA-3B amplified a 721 base pair long fragment and the inner primers IEA-3A and IEA-3B amplified a 167 base pair long fragment. In the verification sample the concentration of cells was reduced to 0.5 x 10⁶ cells/ml before lysis.

The rapid isolation technique was a modification of the detection of early antigen foci or shell vial cell culture technique using a mixture of monoclonal antibodies directed to several different CMV antigens. Two concentrations of leukocytes were used corresponding to 1 x 10⁷ and 2.5 x 10⁷ leukocytes/well.

Definitions of CMV disease

CMV disease was defined according to the 4th International CMV Conference criteria. CMV pneumonia was defined as interstitial pneumonia changes on chest roentgenogram, hypoxia, combined with CMV verification from broncho-alveolar lavage fluid, lung biopsy or autopsy material. CMV gastroenteritis was defined as gastrointestinal symp-

toms combined with CMV identified in biopsy material obtained by upper or lower endoscopy. CMV hepatitis was defined as clinical hepatitis combined with CMV detected in a liver biopsy. CMV encephalitis was defined as symptoms of encephalitis combined with CMV DNA detected in the CSF.

Statistics

The original study design included a clinical endpoint and a virologic endpoint. The clinical endpoint was the proportion of patients who developed CMV disease within 4 weeks of study entry. The study sample size was calculated to require a minimum of 13 evaluable patients based on the clinical endpoint.

The virologic endpoint was the number of patients who reverted from a positive rapid isolation to a negative at the end of the 2 weeks of foscarnet therapy. However, due to the low proportion of patients who were rapid isolation positive at inclusion, the virologic endpoint was for the analysis changed to the proportion of patients developing a positive rapid isolation during therapy thus experiencing a failure of foscarnet to control CMV replication.

Results

Fifteen patients were included in the study. One patient did not fulfill the inclusion criteria of two consecutive positive PCRs and was excluded from the efficacy evaluation. This patient received 4 days of therapy, all subsequent PCR surveillance tests were negative and the patient did not develop any signs of CMV disease. Thus, the efficacy calculations were based on 14 patients.

CMV disease

None of the 14 patients developed CMV disease during the study period of 4 weeks. Furthermore, none of the patients developed CMV disease before day 100 after BMT.

Other herpes virus disease

One patient developed a herpes simplex virus type 2 menigitis diagnosed by PCR on CSF after 9 days of therapy. The foscarnet dosage was then increased to 60 mg/kg three times daily during the remainder of the study period and he recovered without neurological sequelae.

Virologic response to therapy

At study entry 14/14 patients were positive by PCR and 3/14 by either rapid or standard isolation. Information of isolation results was not available on five patients at study entry. At the end of foscarnet therapy five of 13 evaluable patients were negative by PCR at both the lower sensitivity, and the higher sensitivity levels. Twelve of 13 patients were also negative by isolation while information was missing on one patient. However, this patient was negative by PCR at end of therapy. The patient who received an increased
dosage of foscarnet due to HSV-2 meningitis was negative at both sensitivity levels at the end of foscarnet therapy.

In samples drawn between days 16 and 21 after initiation of therapy eight of 14 were negative by PCR at both sensitivity levels and none had become positive in virus isolation. Finally, in samples drawn at the end of the follow-up period, nine of 14 patients were negative by PCR at both sensitivity levels.

Additional antiviral therapy

Three patients received an additional course of antiviral therapy, two patients with foscarnet and one with ganciclovir. The second course was given due to reappearance of CMV DNA after discontinuation of previous therapy. No CMV disease developed in any of these patients.

Toxicity

All patients were closely monitored for side-effects, both for the development of suspected foscarnet-associated symptoms and laboratory abnormalities. No patient had to stop therapy due to side-effects and no serious adverse event was reported. Altogether six patients developed gastrointestinal symptoms during the study period with nausea reported in four, vomiting in three, and diarrhea in two patients. None of these symptoms was graded as severe. Three patients reported headache; none was severe. Finally three patients reported signs of mild urethritis or mild hemorrhagic cystitis that disappeared after discontinuation of foscarnet therapy.

Two of 15 patients developed increased serum-creatinine values. The foscarnet dosage was adjusted according to the protocol specifications and both patients completed the 14 days of therapy. Six of 15 patients developed abnormal serum-magnesium levels, seven patients abnormal serum-potassium levels, seven abnormal serum-phosphate levels, and five abnormal serum-calcium levels. None of the patients needed dosage adjustment of foscarnet due to serum-electrolyte abnormalities. Only one of 15 patients developed leukopenia and none thrombocytopenia during the study period.

Discussion

Pre-emptive therapy is one of two potential strategies that can be used for prevention of CMV disease in allogeneic BMT recipients. The advantage with pre-emptive therapy is that not all patients need to be exposed for antiviral agents with potential toxic side-effects. Previously existing diagnostic techniques such as rapid isolation with early antigen detection has not been sensitive enough and in a study by Goodrich et al.15 11% of the patients developed CMV disease before pre-emptive therapy could be initiated. Recent developments in diagnostic techniques such as antigens or PCR allow earlier initiation of therapy and thereby a reduction in the risk of developing CMV disease.3,5,13

The antiviral agent that has been used most for pre-emptive therapy is ganciclovir (GCV). However, GCV frequently causes leukopenia.12 Foscarnet is an effective anti-CMV agent but its use in allogeneic BMT patients has been hampered by renal toxicity in particular together with other nephrotoxic agents such as cyclosporin.14 Our hypothesis was that if CMV infection could be detected and pre-emptive antiviral therapy initiated earlier a lower dosage of foscarnet could be used without increasing the risk for development of CMV disease. This small pilot trial was therefore designed to test this hypothesis. The chosen dose was 60 mg/kg twice daily which is two thirds of the dose recommended for treatment of established CMV disease. The study design included the option to increase the dose if breakthrough of CMV disease occurred but this option was not used. The results of this trial indicate that foscarnet in a dosage of 60 mg/kg twice daily can be used as pre-emptive therapy in allogeneic BMT patients. Obviously the number of patients in this trial was small. However, the number 0 of 14 patients developing CMV disease is similar to 0 out of Emsch et al.11 who reported that two of 37 patients pre-emptively treated with ganciclovir developed CMV disease.2 It is also similar to our own previous experience of development of CMV disease in three of 28 patients treated with either ganciclovir or full dosage foscarnet.4 Also the proportion of patients (9/14; 64%) who became PCR negative on 2 weeks of foscarnet therapy was similar to our previous experience (78%) with GCV or full dosage foscarnet.4

Our second aim with this pilot study was to evaluate the toxicity of this reduced dosage of foscarnet. The side-effects that were noted in this pilot study such as gastrointestinal symptoms, urethritis, and electrolyte disturbances are well known with foscarnet therapy. The number of patients treated was too low to assess if the side-effects noted on this foscarnet dosage was different from what could be expected on standard dosage foscarnet therapy. However, we believe that the results were also encouraging in this aspect since none of the patients developed significant renal toxicity and all patients could complete the intended therapy course.

Thus, both the efficacy and toxicity results of this small pilot trial indicate that foscarnet in a dosage of 60 mg/kg given twice daily can be used as pre-emptive therapy in allogeneic BMT recipients with early signs of CMV infection. This treatment protocol will be further tested in an ongoing prospective randomized trial comparing GCV and foscarnet by the European Group for Blood and Marrow Transplantation (EBMT).

References


