Brincidofovir is highly efficacious in controlling adenoviremia in pediatric recipients of hematopoietic cell transplant

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Key points

Brincidofovir has superior anti-adenoviral activity and safety profile compared to Cidofovir.

Brincidofovir is highly efficacious in controlling adenoviraemia during lymphopenic phase of HCT.

Summary

Cidofovir is pre-emptively used for controlling adenoviremia and preventing disseminated viral disease in hematopoietic cell transplant (HCT) recipients but does not lead to resolution of viremia without T-cell immune-reconstitution. The lipid conjugated prodrug of Cidofovir, Brincidofovir, has improved oral bioavailability and achieves higher intracellular concentrations of active drug. We present retrospective multicentre data comparing the kinetics of viremia and toxicities following pre-emptive treatment with Cidofovir and Brincidofovir in children and adolescents diagnosed with HCT-related adenoviremia. Forty-one episodes (18=Brincidofovir; 23=Cidofovir) of antiviral therapy were observed in 27 patients. The two groups had comparable immune-reconstitution and viral burden. Major (≥2 log reduction in 2 weeks; *n=13*) and minor (≥1 to ≤2 log reduction in 2 weeks; *n=2*) virological responses were observed in 15 (83%) Brincidofovir episodes compared to only 2 (9%) major virological responses with Cidofovir (*p<0.0001*). Brincidofovir mediated major responses in 9 of 11 Cidofovir-unresponsive patients and resulted in complete responses (CR) despite significant lymphopenia (Brincidofovir *vs* Cidofovir; CR = 13 (80%) *vs* 8 (35%); median lymphocyte count = 320/µl *vs* 910/µl; *p<0.05*). One patient experienced severe abdominal cramps and diarrhoea necessitating interruption of Brincidofovir and none developed nephrotoxicity with Brincidofovir. Thus, Brincidofovir is well-tolerated and highly efficacious in controlling adenoviremia during the lymphopenic phase of HCT.

Introduction

There is no established pre-emptive therapy to control adenoviremia post-HCT, despite a 15% risk of significant viral reactivation[1] and 20 to 80% risk of mortality in disseminated disease.[2] Cidofovir, a monophosphate nucleotide analogue of deoxycytidine competitively inhibits incorporation of deoxycytidine into viral DNA, leading to viral DNA termination and is the only drug readily available to control HCT-related adenoviremia. Although, the use of pre-emptive antiviral therapy/Rituximab has significantly reduced Cytomegalovirus/Epstein Barr virus related morbidity and mortality following HCT, Cidofovir had no comparable impact on the management of adenoviral problems.[3-6] Cidofovir is nephrotoxic,[7] has poor cellular uptake and is unable to mediate complete resolution of viremia in the absence of immune-recovery.[8,9] A lipid-linked derivative of Cidofovir, Brincidofovir (CMX001, Chimerix Inc), has recently been developed. The lipid conjugation improves oral bioavailability and increases intracellular concentration of the active drug. Brincidofovir, unlike Cidofovir, is not a substrate for organic anion transporter 1 in the renal tubules and hence is not nephrotoxic.[10] We therefore compared the efficacy of Brincidofovir and Cidofovir in controlling HCT-related adenoviremia and assessed the toxicity profile in a retrospective UK-wide multicentre paediatric study.

Methods

Definition of significant viremia and exclusion criteria

A total of 333 recipients undergoing allogeneic stem-cell transplant in seven paediatric transplant centres from January 2015 to May 2016 were screened weekly using an adenovirus polymerase chain reaction (PCR) until immune-recovery and cessation of immunosuppression. Patients with significant viremia (adenovirus levels ≥ 1000 copies per mL) on two consecutive occasions were treated with an antiviral therapy. Patients with single PCR positivity and/or PCR level below the limit of sensitivity were excluded.

Treatment of adenoviremia

Withdrawal of immunosuppression was commenced immediately in patients with significant viremia and without graft-versus-host disease (GVHD).[11] The standard of care pre-emptive therapy was either Cidofovir 5 mg/kg weekly for two consecutive weeks followed by fortnightly dosing or 1 mg/kg three times weekly. Patients with pre-existing renal impairment, those that developed renal impairment on Cidofovir or those not responding to two weeks of Cidofovir were considered for treatment with 2 mg/kg twice weekly Brincidofovir through an expanded access program.

Assessment of virological response and its relationship with lymphocyte reconstitution

Major and minor virological responses were defined as two-log and one-log reduction of viral load respectively within two weeks of starting antiviral therapy. Treatment failure was defined as no reduction in viral load within 2 weeks of commencing antiviral therapy. The relationship between virological response and lymphocyte reconstitution was also studied.

The peak viral load and the total lymphocyte count at commencement of antiviral therapy were recorded (supplemental figure 1). The area under the curve (AUC) of viral load against time on therapy was recorded during antiviral therapy (supplemental figure 2).

Toxicity profile

The common terminology criteria for adverse events version 4.0 was used to assess the toxicity of antiviral therapy.[12]

Statistical analysis

Fischer's exact test and unpaired *t*-test was used to analyse categorical variables and continuous variables respectively.

Results and discussion

Adenoviremia was reported in 47 (14.1%) patients and significant viremia requiring antiviral treatment was noted in 27 patients (8.1%). Five patients had adenoviral disease at onset of viremia. The transplant characteristics of patients with significant viremia are summarized in supplemental table 1.

Patients were treated with Cidofovir only (*n=9*), Cidofovir followed by Brincidofovir (*n=12*), Brincidofovir only (*n=4*) and Cidofovir followed by adenovirus-specific cytotoxic lymphocytes (*n=2*). A total of 18 Brincidofovir episodes in 16 patients and 23 Cidofovir episodes in 23 patients were observed. Thirteen Brincidofovir episodes received concurrent steroid treatment for GVHD (2 mg/kg of methylprednisolone; *n=8*, 1 mg/kg of methylprednisolone; *n=5*) and twelve Cidofovir episodes received such treatment (2mg/kg of methylprednisolone; *n=6*, 1 mg/kg of methylprednisolone; *n=6*). Patients treated with Cidofovir *versus* Brincidofovir were comparable for use of serotherapy and HLA matching (supplemental table 2). The median time from HCT to initiation of CDV vs BCV was 28 days (interquartile range: 17 to 43) vs 53 days (interquartile range: 29 to 78) respectively. However, the two groups had comparable compromise of immune-reconstitution and similar viral burden (supplemental figure 1).

Brincidofovir mediated major and minor virological responses in thirteen (72%) and two episodes (11%) respectively. In contrast, Cidofovir mediated major virological responses only in 2 episodes (9%) (*p<0.0001*; Figure 1(a), 1(b), 2(a) and supplemental figure 3; supplemental table 3). Thirteen patients (80%) cleared viremia with Brincidofovir compared to 8 patients (35%) with Cidofovir. The median time to clearance of viremia with Brincidofovir was 4 weeks (range: 2 to 9) compared to 9 weeks (range: 3 to 15) with

Cidofovir (*p<0.005*). Major virological responses to Brincidofovir were also apparent in 9 of 11 Cidofovir-unresponsive episodes (Figure 2(a) and 2(b)).

Virological responses following the use of Cidofovir have been described to be mediated by immune-recovery.[8,9] Therefore, to study the confounding effect of immune-reconstitution on virological responses, we compared the circulating lymphocytes at the initiation of Brincidofovir and at 2-log reduction in viral load. The median lymphocyte count prior to Brincidofovir was 250/µl (range: 0 to 4100) compared to 320/µl (range: 0 to 4300) on 2-log reduction in the viral load (*p=NS*). Ten of the 15 Brincidofovir responses occurred despite ≥1mg/kg methylprednisolone (supplemental figure 4(a) and 4(b)). In addition, at complete resolution of viremia, the median lymphocyte count in the Brincidofovir group (*n=15*) was 320/µl (range: 0 to 3000) compared to 910/µl (range: 380 to 2000) in the Cidofovir group (*n=8*) (Figure 1(b)). Thus, majority of patients were responsive to Brincidofovir despite having an absolute lymphocyte count ≤ 300/µl, which has previously been described as associated with mortality from adenoviral infection post-SCT[11,13] and despite concomitant steroid treatment for GVHD (*p<0.05*).

We determined the relevance of virological responses and the clinical risk of disseminated viral infection. In this study cohort, 2 patients treated with Cidofovir died of disseminated adenoviral infection. The AUC of viral load was significantly higher in these patients with virus-related mortality than those who died because of other causes (*p<0.02*) and those who did not suffer mortality (*p<0.0001*; supplemental figure 2). It is noteworthy that the AUC of viral load in the 4 patients treated with Brincidofovir as a first line drug was significantly lower than the AUC of virus-related mortality (*p<0.0004*). This is an important observation

and requires further studies to demonstrate the role of Brincidofovir in reducing the risk of adenovirus-related morbidity and mortality.

Adenovirus-related complications can also be reduced by adenovirus-specific T-cell therapy using IFNγ-capture technology. This technology allows rapid collection of adenovirus-specific T cells from the donor, however a significant investment in infrastructure and expertise is required.[14,15] Further, in a recent study of adenovirus-specific Th1 cell therapy,[14] only 4 (15%) of 26 patients were major responders and a quarter of patients were non-responders, all of whom died. In addition, adenovirus T-cell therapy did not prevent mortality if organs were involved prior to T-cell transfer. In another study of five paediatric HCT recipients, only one patient achieved major virological response.[15] These observations emphasize the importance of an effective pre-emptive therapy to prevent progression to adenoviral disease.

There were 3 non-responders in our study of which one patient had stage 4 gut GVHD. These failed responses may be because of differences in gut absorption and hence drug pharmacokinetic studies may help to understand failed virological responses and, an intravenous formulation may improve virological responses in patients with gut GVHD.[16] One possible reason for failure to respond is acquisition of adenoviral resistance to Brincidofovir, although previous trials have shown infrequent occurrence of Brincidofovir resistance.[17]

Brincidofovir was well-tolerated and only one patient required interruption of Brincidofovir after four weeks because of severe abdominal cramps and diarrhoea. Brincidofovir-mediated diarrhoea was reported as a frequent side-effect in the phase 3 double-blind placebo controlled trial for preventing CMV infection[18] leading to eight-fold increase in use

of steroids in the Brincidofovir arm due to presumed diagnosis of gut GVHD. Hence, it is important to distinguish Brincidofovir toxicity from gut GVHD and virus-associated diarrhoea in post-HCT patients. Brincidofovir was not observed to mediate nephrotoxicity. In contrast, 9 (39%) of 23 patients treated with Cidofovir developed mild to moderate nephrotoxicity as previously described (supplemental figure 5).[7]

In conclusion, we demonstrate that Brincidofovir has a good safety profile and excellent anti-adenoviral activity, even during the lymphopenic phase of HCT. Therefore, Brincidofovir may be a useful pre-emptive therapy for HCT-related adenoviremia. Despite the limitations of a retrospective study, this is the largest reported study of the use of Brincidofovir for adenovirus to date and reports contemporary experience from multiple centres. Further prospective studies of this agent are now indicated to confirm the anti-adenoviral effect of Brincidofovir in the absence of virus-specific responses.

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Contributing Authors

P.H., P.A., P.V., and R.W. designed research; P.H. collated the data and performed statistical analysis; C.O., P.S., H.D., M.S., T.P., K.P., S.H.L., S.L., C.S., A.G., J.S., K.R. contributed the data and P.H., and R.W. wrote the manuscript.

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