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Acyclovir Prophylaxis of Oral Herpes Virus During Bone Marrow Transplantation

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Oropharyngeal shedding of herpes viruses (herpes simplex, cytomegalovirus) was assessed in patients on standard acyclovir prophylaxis during bone marrow transplantation (BMT) to determine the frequency of viral shedding and to assess possible oropharyngeal complications that may be associated with viral reactivation in these patients. We conducted a prospective assessment of 83 patients receiving BMT. Patients were evaluated weekly and oral surveillance cultures were completed. Shedding of herpes simplex virus (HSV) was detected in the oropharynx of 2.9% of seropositive patients on prophylactic acyclovir, and only one case of clinical oral herpetic infection was seen. Cytomegalovirus (CMV) was cultured from the oropharynx in 13.3% of CMV seropositive patients provided with prophylactic acyclovir, but no oropharyngeal lesions were attributed to CMV reactivation. No correlation was seen between HSV and CMV pretransplant serology and severity of oral mucositis and acute graft versus host disease. No effect on time to engraftment was detected. This study supports the continuing use of acyclovir prophylaxis in HSV seropositive patients receiving BMT. Acyclovir prophylaxis was effective in preventing viral shedding in all but 2.9% of patients, and only one case of clinical infection was diagnosed. The frequency of CMV shedding was approximately four times that of HSV; however, no oral lesions were attributed to CMV. Copyright © 1996 Elsevier Science Ltd

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INTRODUCTION

Reactivation of latent herpes viruses, specifically herpes simplex virus (HSV) and cytomegalovirus (CMV), is common in bone marrow transplantation (BMT) [1–11]. Indeed, up to 80–90% of HSV seropositive patients will reactivate the virus during treatment [1–7, 10]. CMV causes significant morbidity and mortality in BMT patients, including pneumonitis, gastrointestinal ulceration, retinitis and other manifestations [6]. CMV seropositive patients, and seronegative patients who acquire CMV from the donor or via blood products, are at risk.

The prophylactic use of acyclovir has become routine to prevent reactivation of latent herpes viruses in BMT [1–3, 5, 6, 12–14]. While prevention of reactivation of CMV by acyclovir has been reported, acyclovir does not control active infection [6, 12, 14]. Preventive strategies for CMV infection include the use of anti-viral drugs for CMV-positive marrow recipients and for seronegative patients who receive marrow from a seropositive donor, using acyclovir and more recently ganciclovir [6, 12–20]. Additional strategies include passive immunity with CMV gammaglobulin products. The use of CMV-negative blood products in seronegative donor–recipient pairs is an effective preventative strategy in eligible patients.

Reactivation of herpes viruses may occur, even when appropriate anti-viral agents are used for suppression [7, 17, 21–39]. In addition, the use of acyclovir during BMT may blunt the specific immune response to HSV, possibly due to delayed exposure of the patient to antigens of HSV, leading to increased frequency of HSV recurrence when acyclovir is discontinued [1, 2]. However, reactivation of HSV following recovery of the white cell counts may result in less severe outbreak of infection.

This study was a systematic follow-up of BMT patients who had received acyclovir prophylaxis for HSV and CMV, to determine the frequency of oropharyngeal HSV and CMV shedding and clinical infection due to these viruses and to assess the relationship of viral infection to oral complications following BMT [21–23].

METHODS

Clinical methods

83 consecutive BMT patients who received BMT between December 1987 and December 1989 were assessed. Informed

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consent was obtained. The oral cavity was examined daily by nursing and medical practitioners, and weekly by dental providers. Oral viral cultures were conducted weekly and when lesions suspicious of herpes virus infection were seen. Lesions at other sites were cultured based upon clinical appearance and suspicion of the clinician.

Of the 83 patients, 50 were male and 33 female. The median age was 34 years (range 14–55). The medical diagnoses of the patients were either acute myelogenous leukaemia (AML) or chronic myelogenous leukaemia (CML) (Table 1). 41 patients were treated with autologous BMT (auto-BMT), and 42 with allogeneic BMT (allo-BMT) (Table 1). The pre-transplant conditioning regimen included total body irradiation (TBI) in 29% of patients (Table 2). Graft versus host disease (GVHD) prophylaxis was provided to 23 patients using cyclosporine and methotrexate with and without folinic acid [40].

During this period the HSV and CMV serostatus of all patients was determined prior to treatment. All HSV seropositive patients were provided with prophylactic acyclovir (usual dose 250 mg/m^2 i.v. q8h); all patients who were CMV seropositive received high-dose acyclovir prophylaxis (at a planned dose of 500 mg/m^2 i.v. q8h) (Table 3).

The clinical criteria used for the diagnosis of HSV in BMT patients have been described [4, 7, 41–44]. Briefly, in the early period following conditioning for BMT, severe haemorrhagic mucositis may be associated with HSV and later, following transplant, vesicles or ulceration generally present as 1–5-mm rounded lesions involving keratinised tissues (lip, gingiva, palate, lateral–dorsal tongue). When lesions become progressive, large ulcerations (1 cm or greater) may develop and involve any mucosal surface. A nonspecific mucositis grade was assigned by the medical staff using a modified National Cancer Institute scale [45]. In this system, grade 1 toxicity was pain without ulcers; grade 2 was painful ulceration; grade 3 was ulcerative mucositis requiring narcotic analgesia for less than 2 weeks; and grade 4 represented ulcerative mucositis requiring narcotic analgesic for more than 2 weeks. Acute GVHD was graded using the Seattle and Minnesota criteria [46, 47].

Table 1. Patient characteristics

Diagnosis	No.	Donor source	No.
Acute lymphocytic leukaemia	6	*Allogeneic*	
Acute myelogenous leukaemia	15	Matched sibling	30
Chronic myelogenous leukaemia	23	Mis-matched sibling	3
Hodgkin's disease	12	Unrelated donor	9
Non-Hodgkin's lymphoma	13		
Multiple myeloma	6	*Autologous*	
Refractory anaemia with excess blasts in transformation	3	Unpurged	21
		4 HC purged	16
Testicular carcinoma	4	Long-term culture	3
Ovarian tumour	1	Peripheral stem cell	1

Table 2. Pretransplant conditioning regimen

Conditioning regimen	No.
Total body irradiation (TBI)-containing	
Cytoxan + TBI	7
Etoposide + cytoxan + TBI	13
Etoposide + TBI	4
Non-TBI-containing	
Cyclophosphamide + carmustine + etoposide	13
Bisulphan + cyclophosphamide + melphalan	8
Carboplatin + busulphan	6
Carboplatin + etoposide	5
Other (single agent + melphalan)	2

Laboratory study

Antibodies to HSV and CMV were assayed by enzyme linked immunoassay (ELISA) (Behring: Marburg, Germany) and by complement fixation (CF) according to standard procedures [48]. The antigens for CF were supplied by M.A. Bioproducts (Walkersville, Maryland, U.S.A.). Seropositivity for CF was defined as a titre of $\geqslant 1:4$ as this value correlated with positive findings on enzyme immunoassay and Western blot [49]. The specimens for viral culture were collected on dacron swabs and transported to the laboratory in viral transport medium on ice. For viral isolation the transport vial was vortexed and 0.2 ml of medium was inoculated into each of two tubes of human diploid fibroblast cultures and examined for characteristic cytopathic effects for a maximum of 4 weeks. Positive results were confirmed by direct immunofluorescence with monoclonal antibodies for HSV-1, HSV-2 and CMV (Syva Corporation, Palo Alto, California, U.S.A).

Statistical analysis. Data were entered on D-Base III+ (Ashton-Tate, Torrence, California, U.S.A.). The potential effect of serology on engraftment was examined using the Mann–Whitney test. All other comparisons were made using Pearson's Chi-square.

RESULTS

The median length of stay was 50 days (range 7–127, mode 57 days). 51 (61.4%) patients were discharged following BMT; however, 32 (38.6%) patients succumbed to their presenting disease or a complication of treatment.

Positive HSV serology was identified in 69 patients (83.1%) prior to transplantation. 37 (44.6%) provided a prior history of herpetic infection, while 19 were unsure of prior infection. Prior to chemotherapy, 2 cases of HSV culture-confirmed oral lesions were detected and acyclovir was initiated. A total of 77 patients were placed on acyclovir to suppress infection by HSV or CMV, 43 patients (51.8%) received 250 mg/m^2 q8h and 34 patients 500 mg/m^2 q8h. In 3 of these patients, lesions suggestive of HSV were identified during admission and HSV was recovered in two patients, confirming the clinical diagnosis. HSV was recovered in one patient who did not have oral ulcerations. One of 7 patients who was seronegative at admission and not placed on acyclovir prophylaxis developed lesions consistent with HSV and became HSV culture positive during BMT. In 2 cases, HSV was cultured from the perineal/anal area.

CMV serology was positive in 49 (59.0%), negative in 33 and not done in 1 patient. CMV was recovered in 6 patients, with 3 cases from the gut, 2 from the bronchus and 1 in oral culture. No clinical findings of oral CMV infection were identifed. 5 of the 6 patients were CMV seropositive upon admission and had received prophylaxis using high-dose acyclovir, while one patient seropositive only for HSV had been provided with acyclovir at low dose only.

Table 3. Dose of acyclovir during BMT admission

Initial acyclovir dose			Change in acyclovir dose		
Acyclovir dose (mg/m^2/tid)	No. of patients	%	Acyclovir dose (mg/m^2/tid)	No. of patients	%
None	6	7.2			
250	43	51.8	250	13	22.4
500	34	41.0	300	5	8.6
			350	3	5.2
			400	2	3.4
			450	3	5.2
			500	16	27.6
Totals	83	100		42	100

Acyclovir dosage was continued unchanged during the admission in 33 patients, but was changed in 44 patients. The change in dose represented a modest reduction in dose for the patients (mean 366 to mean 309 mg/m^2/q8h), usually due to renal insufficiency.

Non-specific oral mucositis was seen in 77 (93%) patients, and was grade 1 in 18, grade 2 in 37, grade 3 in 17 and grade 4 in 5. The patients who received TBI had a more severe grade of mucositis (mean of 2.35) compared with those on chemotherapy alone (mean of 1.83). Acute GVHD (aGVHD) was diagnosed in 12 (14.5%) patients. The commonest sites of aGVHD were the liver (6) and skin (5). Oral aGVHD was diagnosed in 1 patient (8.3% of the patients who developed aGVHD). The severity of aGVHD was assessed as grade 1 in 5, grade 2 in 4, grade 3 in 2 and grade 4 in 1. No correlations between pre-BMT HSV serology, culture for HSV, oral herpetic lesions or oral mucositis were seen. Also, no correlation between HSV or CMV serology and the development of GVHD and the time to engraftment was seen.

DISCUSSION

Herpes simplex reactivation in BMT has been well recognised as an important cause of oral ulceration that may result in significant morbidity. After BMT, a high proportion of HSV seropositive individuals not given acyclovir prophylaxis will shed HSV [1–7, 10]. Systemic infection due to colonising oral flora including bacteria and fungi has been associated with the presence of mucosal ulceration [50–53]. Because of the risk of systemic infection in ulcerative mucosis due to oral flora, and the finding that reactivation of HSV is predictable during BMT, prophylaxis against HSV reactivation with acyclovir has become routine. As a result, there has been considerable change in the nature and frequency of the oral complications during BMT. However, thorough examination, and clinical suspicion continue to be of importance as the use of acyclovir may not prevent shedding of HSV in all cases, nor does it guarantee that clinical disease due to HSV is always prevented. This may be due to selection of acyclovir-resistant virus, or to breakthrough of HSV in spite of acyclovir use during BMT.

In a retrospective analysis of 627 BMT patients from Seattle, 37% developed oropharyngeal HSV [7]. Only 2 of these patients who developed oral HSV were seronegative pretransplant [7]. An increased risk of HSV reactivation was seen in patients with higher pretransplant anti-HSV titres, in patients who received TBI, in patients with a pretransplant diagnosis of leukaemia and in those who received transplantation when not in remission [7]. In a previous prospective study of HSV in 29 leukaemic patients, we found that HSV reactivation occurred when HSV antibody was positive [41]. We identified one case where asymptomatic shedding of HSV occurred in a patient who was seronegative and not provided acyclovir; otherwise, we confirmed the findings that HSV infection in BMT is essentially due to reactivation of latent virus. We isolated CMV from oral cultures in 16.7% of cases where CMV was identified (1 of 6 cases), but no oral lesions were attributed to CMV.

A number of studies have evaluated the effectiveness of acyclovir for antiviral prophylaxis. In a large placebo-controlled study in BMT, acyclovir prevented HSV and varicella zoster virus (VZV) but not CMV infection when the drug was used on a long-term basis [12]. A review of consecutive BMT patients on acyclovir prophylaxis revealed oral soft-tissue lesions in 13% compared to 60% in historic controls not receiving acyclovir prophylaxis [54]. In the study reported here, no oral lesions were HSV culture positive while on acyclovir, with 2 cases demonstrating HSV-associated lesions after discontinuing acyclovir. Another study attempted to follow the oral mucositis of 59 patients who received prophylactic acyclovir [8]. While three-quarters of patients developed ulcerative mucositis, HSV was not identified on culture, indicating that viral breakthrough did not occur in these patients. Others have reported shedding of HSV in up to one-half of BMT patients receiving acyclovir in different dosages by oral or intravenous routes [21, 22, 55, 56]. In our study, we found that shedding of HSV occurred in the oral cavity of 2 patients who were seropositive prior to BMT and who were given acyclovir prophylaxis and in one case who was seronegative prior to BMT and not provided with acyclovir. Therefore, viral shedding was seen in 2 of 69 (2.9%) seropositive patients despite the use of prophylatic acyclovir during BMT. In 1 case, oral lesions due to HSV were seen (1.5%) due to failure of acyclovir prophylaxis (500 mg/m^2, q8h).

Resistance of HSV to acyclovir has been reported in animal studies [30–32] and increasingly in immunocompromised patients [7, 21–23, 30, 34–39, 57, 58]. Studies have demonstrated that from 5 to 14% of immunocompromised patients treated with acyclovir may have resistant HSV that may cause clinical disease. The most common mechanism of acyclovir resistance in HSV is deficiency of thymidine kinase, although these strains remain susceptible to antivirals that act only on

DNA polymerase (e.g. foscarnet). However, DNA-polymerase mutants have also been identified that are resistant to acyclovir as well as to all current nucleoside analogue antivirals [13, 21, 23, 57]. Prophylaxis may alter the risk of development of viral resistance; as prophylaxis is not always effective, mixed populations of sensitive and resistant viruses may co-exist or resistant virus may develop due to mutation [21, 31, 32, 57, 58]. Because of the risk of clinical disease due to acyclovir-resistant HSV in immunocompromised patients, careful clinical assessment and rapid detection of resistance are necessary in order to permit the provision of effective antiviral therapy [38].

In a multi-centre study of 310 BMT recipients at risk of CMV infection, patients were randomised to received i.v. acyclovir (500 mg/m^2, tid) for 1 month followed by oral acyclovir (800 mg qid) or i.v. acyclovir for 1 month followed by placebo or low-dose oral acyclovir (200 or 400 mg qid) for 1 month followed by placebo [59]. This study showed that high-dose i.v. acyclovir was of value in prophylaxis of CMV infection. Also, no effect upon GVHD was seen between patient groups. In the study reported here, CMV was cultured from 6 cases, 5 of whom were CMV seropositive prior to transplant. CMV was recovered from the oral cavity despite the use of acyclovir in one case. Thus, in 6 of 49 (13.3%) CMV seropositive patients reactivation and shedding of CMV was seen despite acyclovir prophylaxis. Recent studies have shown the effectiveness of another antiviral agent—ganciclovir (DHPG)—in preventing CMV infection post-BMT, although haematological toxicity is a concern and CMV resistance to ganciclovir has been reported [13, 16, 17, 19, 20, 24–29, 60].

The effect of prophylactic acyclovir on oral mucositis was evaluated in 59 BMT patients; 46 developed ulcerative mucositis, but HSV was recovered in only one case [8]. There was no evidence of an effect of HSV serostatus on the severity of ulcerative mucositis. The authors concluded that prophylactic acyclovir was effective in preventing HSV reactivation, and that HSV was probably not a major aetiologic agent of mucositis [8]. This finding is confirmed in our study. We did not identify a correlation between the development of oral mucositis and HSV reactivation, indicating the effectiveness of acyclovir in preventing reactivation of HSV. The patients who received TBI had a more severe grade of mucositis (mean of 2.35) than those who were conditioned with chemotherapy (mean grade of 1.83). Therefore, the conditioning regimen appeared of importance in the severity of mucositis, with the addition of TBI leading to increased severity of mucositis. Bostrom and coworkers reported that high IgG titres for HSV in the BMT recipient was statistically correlated with aGVHD ($P=0.05$) and that HSV seropositivity in the donor and donor mononuclear cell reactivity to HSV antigen was associated with an increase in Grade II–III GVHD ($P=0.004$) [61]. In our study we documented aGVHD in 21.7% of patients. The commonest sites of aGVHD were the liver and skin. Oral aGVHD was diagnosed in one patient. We did not demonstrate a correlation between HSV serology and the development of aGVHD. No correlation between HSV or CMV serology and time to engraftment was seen.

We conclude from the results of this study that parenteral acyclovir significantly reduces, but does not completely eliminate, HSV shedding and oral lesions following BMT. In 2.9% of cases HSV was identified in oral secretions following acyclovir prophylaxis and in one case (1.5%) clinical HSV infection occurred. CMV shedding was identified in 12% of seropositive patients (1 of 6 cases in oral secretions), no oral lesions were attributed to CMV. When acyclovir prophylaxis is used, oral mucositis relates to the conditioning regimen. No relationship between pretransplant HSV or CMV serology and time to engraftment and aGVHD is seen when acyclovir prophylaxis is used. We concur with the current practice of suppressing HSV reactivation with acyclovir in HSV seropositive patients during BMT.

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