A Non-Randomized Comparative Study Using Different Doses of Acyclovir to Prevent Herpes Simplex Reactivation in Patients Submitted to Autologous Stem Cell Transplantation

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The reactivation of Herpes Simplex virus (HSV) occurs in 70% to 80% of patients submitted to autologous stem cell transplantation (ASCT); it increases the severity of chemotherapy-induced mucositis. Therefore, the use of acyclovir in ASCT patients is considered standard practice. However, the minimum dose needed to prevent reactivation is a matter of debate. We compared two doses of acyclovir in a non-randomized fashion in 59 patients submitted to ASCT: 32 patients received a dose of 125 mg/m^2 IV every six hours and the subsequent 27 patients received a dose of 60 mg/m^2 IV every six hours. Viral excretion was evaluated through weekly viral culture of oral swabs. Grade 4 mucositis was more frequent in Group 1 (p= 0.03). The reactivation rates in Groups 1 and 2 were 9% and 4%, respectively (p= 0.62, 95% confidence interval -7 – 18). Prophylaxis with reduced doses of intravenous acyclovir seems to be as effective as a higher dose in inhibiting HSV reactivation, with a significant reduction in cost. Prospective randomized studies are needed to confirm our conclusions.

Key Words: Acyclovir, prophylaxis, stem cell transplantation.

Infection by Herpes Simplex virus (HSV) in patients submitted to autologous hematopoietic stem cell transplantation (ASCT) occurs in 70 to 80% of seropositive individuals; it is due mostly to viral reactivation [1]. Mucocutaneous lesions indistinguishable from chemotherapy-induced mucositis are the commonest clinical presentation, but other manifestations may occur, including esophagitis, pneumonia and disseminated infection [2]. The use of acyclovir as prophylaxis against HSV reactivation is considered standard in the care of ASCT patients during the period of neutropenia [3]. However, since acyclovir is costly, this drug accounts for a substantial proportion of the total cost of antimicrobial agents. Therefore, procedures to reduce the dose of acyclovir without compromising its efficacy could have a significant and favorable impact on the final cost of transplantation.

Randomized studies using different doses of acyclovir in neutropenic patients have been performed [2,4-7]. The rates of HSV reactivation varied between 0 and 20.8%, using doses ranging from 62.5 mg/m^2 every four hours to 250 mg/m^2 every eight hours. We compared two doses of acyclovir as prophylaxis against HSV disease in ASCT recipients in a non-randomized fashion to determine if a lower dose (60 mg/m^2 every six hours) would be as effective as a higher dose (125 mg/m^2 every six hours).

Materials and Methods

During a 36-month period (February 1997 to February 2000), 59 consecutive patients were

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submitted to ASCT at the University Hospital, Universidade Federal do Rio de Janeiro. Patients were eligible to participate in the study if they had not received acyclovir within 14 days of admission and if they signed an informed consent. Prophylactic acyclovir was started within five days of the initiation of the conditioning regimen, and discontinued after neutrophil engraftment (>500 neutrophils/mm^3 for at least three consecutive days) or after disappearance of any clinical sign of mucositis. The subjects were studied consecutively and allocated to one of the two arms in a non-randomized fashion. The first 32 patients (Group 1) received acyclovir at a dose of 125 mg/m^2 intravenously every six hours (February 1997 to January 1999) and the following 27 patients (Group 2) received acyclovir at a dose of 60 mg/m^2 intravenously every six hours (February 1999 to February 2000).

Evaluation of viral shedding was performed at baseline and once a week, until neutrophil engraftment or resolution of mucositis. Three sterile swabs were rubbed in three distinct areas (right and left jugal mucosa, and the mucosa below the lower incisors); they were then transported in an appropriate viral transport medium. The tubes were immediately sent to the Virology Laboratory of the hospital and processed. Viral isolation was performed according to standard procedures [8]. Samples with cytopathic effect were identified for HSV serotype by direct immunofluorescence [9], using a monoclonal antibody for each serotype. Patients who presented viral reactivation during prophylaxis were considered to have failed, and they received acyclovir at a dose of 250 mg/m^2 every eight hours.

We evaluated the patients daily for the presence and intensity of mucositis. The following data were collected prospectively: age, gender, underlying disease and its status at the time of ASCT, conditioning regimen, history of previous infection by HSV (presence, time elapsed from the first documentation of HSV, frequency of relapses, previous use of antiviral drugs and time elapsed since the last use of an antiviral agent).

Until 1998, empirical antibiotic therapy consisted of ceftazidime plus amikacin; vancomycin was added empirically to persistently febrile patients. From January 1998, cefepime was given as empirical therapy, and vancomycin was given only in special situations [10]. Amphotericin B was started empirically after six days of persistent fever, or whenever patients who had become afebrile developed a new fever, provided that no signs of bone marrow recovery were present.

Fever was defined as an axillary temperature >38°C and neutropenia as a neutrophil count <500/mm^3. Fever during the period of neutropenia was classified according to the Immunocompromised Host Society criteria [11] as fever of unknown origin, microbiologically documented, with or without bacteremia, and clinically documented. The presence of mucositis was not considered per se a documentation of infection. HSV reactivation was defined as at least one swab sample positive for HSV. Mucositis was classified according to the World Health Organization toxicity scale as follows: grade 0, no mucositis; grade 1 painless ulcer or erythema; grade 3, erythema, edema and painful ulcers, but ability to eat, and grade 4, need for parenteral or enteral support [12].

The analysis of data was performed on an intention-to-treat basis and included all patients who were enrolled. The outcome variable for the comparison between the two groups was the HSV reactivation rate during acyclovir prophylaxis. Assuming that the rate of HSV reactivation in patients receiving acyclovir at a dose of 125 mg/m^2 every six hours is approximately zero, we estimated a 30% reactivation rate as the worst acceptable result with a dose of 60 mg/m^2 every six hours (we considered that if a lower dose of acyclovir was not effective, the reactivation rate would be similar to that reported in the placebo arms of previously-published randomized studies). With an alpha error of 5% and a beta error of 20%, 27 patients were needed in each group.

The Fisher's exact test (two-tailed) or the Chi-square test was used for the comparison between dichotomous variables. Continuous variables were compared by the Wilcoxon test. The 95% confidence interval (95% CI) for the differences between proportions was also calculated. P values ≤ 0.05 were considered statistically significant. All analyses were performed using the Epi Info 6-04b software (September 1997; CDC, Atlanta, GA, USA).

Results

Fifty-nine patients were included in the study. Thirty-two received intravenous acyclovir 125 mg/m^2 every six hours (Group 1) and 27 received acyclovir at the dose of 60 mg/m^2 every six hours (Group 2). There were no significant differences in age, gender, underlying disease, conditioning regimen, source of stem cells and previous history of HSV disease between the two groups (Table 1), although there were slightly more patients with Hodgkin's disease in Group 2 (30% *vs.* 12%, 95% CI -3 – 38, p= 0.10), and more patients with non-Hodgkin's lymphoma in Group 1 (31% *vs.* 15%, 95% CI -4 – 37, p= 0.14). In addition, there were nine patients in Group 1 with grade 4 mucositis at baseline, compared to none in Group 2 (95% CI -1 – 19, p= 0.15). The use of antibacterial prophylaxis was also more frequent in Group 1 (22% *vs.* 4%, 95% CI 2 – 34, p= 0.059). All patients received fluconazole as antifungal prophylaxis.

Thirty of the 32 patients in Group 1 (94%) and 26 of the 27 patients in Group 2 (96%) developed fever (p= 1.0). Ceftazidime was the main beta-lactam antibiotic used in Group 1 (15 patients, 50% of the febrile episodes) and cefepime was used in 25 of the 26 febrile patients in Group 2 (p= 0.003). The classification of the febrile episodes was not significantly different between the two groups (Table 2). Likewise, there were no significant differences in the median duration of antibiotic therapy (10.5 [range 1 – 22] *vs.* 10 [range 4 – 25], p= 0.72, for Groups 1 and 2, respectively), and success of the empirical antibiotic therapy (47% in Group 1 and 41% in Group 2, 95% CI -19 – 31, p= 0.74). Although the use of empirical antifungal therapy was twice as frequent in Group 1 (31% *vs.* 15%, 95% CI -4 – 40), the difference was not significant (p= 0.12).

Twenty-two patients of Group 1 (69%) and 17 patients of Group 2 (63%) presented mucositis (95% CI -18 – 30, p=0.64). Grade 4 mucositis was observed in six patients in Group 1 and in no patients in Group 2 (95% CI 5 – 32, p= 0.03). The median duration of fever was somewhat longer in Group 1 than in Group 2 (5 days [range 1 – 11] *vs.* 3 days [range 1 – 13], p= 0.06). The duration of neutropenia was significantly longer in Group 1 (11 days [range 8 – 26] *vs.* 8 days [range 5 – 31], p<0.01).

Three patients (9%) in Group 1 and one patient (4%) in Group 2 had reactivated HSV during the study (95% CI -7 – 18, p= 0.62). The relative risk of HSV reactivation in Group 2 was 0.40 (95% CI 0.04 – 3.58).

Three patients died during neutropenia: one in Group 1 and two in Group 2 (95% CI -16 – 7, p= 0.59). None of these patients presented clinical signs of disseminated HSV disease.

Acyclovir was given for a median of 17.5 days in Group 1 and 15 days in Group 2 (p= 0.08). No patient withdrew from the study and no adverse effects attributable to acyclovir were observed.

Discussion

The strategy of defining the smallest effective dose of acyclovir in the prophylaxis of HSV reactivation in immunosuppressed patients has been tested since 1981, when Saral et al. [4] conducted a double-blind randomized trial on 20 seropositive recipients of bone marrow transplants using 250 mg/m^2 IV every 8 hours; the study by Angelopolus et al. [7] established that the dose of 62.5 mg/m^2 IV every four hours was as effective as higher doses in patients with acute myeloid leukemia in induction remission. We used smaller doses than previously tested, and we did not observe an increase in the reactivation rate.

The duration of neutropenia was longer in patients in Group 1. This could be due to the fact that all patients in Group 2 received peripheral blood stem cells, whereas three patients in Group 1 received bone marrow stem cells. ASCT with peripheral blood stem cells is associated with a significant reduction in the duration of neutropenia [13].

An intriguing observation of this study was the higher proportion of patients with severe mucositis in Group 1. At baseline, there were three patients with grade 4 mucositis in Group 1 and none in Group 2. Since the conditioning regimens were well balanced, it is not likely

Table 1. Characteristics of the groups of patients submitted to autologous stem cell transplantation

Characteristic	Group 1 (n=32)	Group 2 (n=27)	95% CI	P
Age, median (range)	44.5 (8 – 63)	48 (11 – 65)		0.39
Gender Male:Female	15:17	14:13	-31 – 21	0.45
Underlying disease, n (%)				
Hodgkin's disease	4 (12)	8 (30)	-38 – 3	0.10
Non Hodgkin's lymphoma	10 (31)	4 (15)	-4 – 37	0.14
Multiple myeloma	15 (47)	11 (34)	-19 – 31	0.64
Others	3 (10)	4 (21)	-22 – 11	0.69
Conditioning regimen, n (%)				
CBV	14 (44)	11 (41)	-22 – 28	0.82
Melphalan 200 mg/m^2	15 (47)	11 (41)	-19 – 31	0.64
Other	3 (9)	5 (18)	-27 – 9	0.45
Source of Progenitor cells, n (%)			-1 – 19	0.15
Bone marrow	3 (9)	0		
Peripheral Stem cells	29 (91)	27 (100)		
Time to start G-CSF, n (%)			-37 – 12	0.23
Day +1	9 (28)	11 (41)		
Day +5	23 (72)	16 (59)		
Previous herpes labialis, n (%)	4 (12)	4 (15)	-20 – 15	0.54
Mucositis on the first evaluation, n (%)	10 (31)	14 (52)	-45 – 4	0.90
Mucositis Grade 4 on the first evaluation, n (%)	3 (9)	0 (0)	-1 – 19	0.15
Antibacterial prophylaxis, n (%)	7 (22)	1 (4)	2 – 34	0.059

95% CI = 95% confidence interval; CBV = cyclophosphamide, BCNU, etoposide; G-CSF = granulocyte colony-stimulating factor.

Table 2. Classification of the febrile episode in Groups 1 and 2

	Group 1 n=32 (%)	Group 2 n=27 (%)	95% CI	P
Patients with fever	30 (94)	26 (96)	-14 – 8	
Fever of unknown origin	10 (33)	7 (27)	-18 – 28	0.60
Bacteremia	11 (37)	10 (38)	-27 – 22	0.89
Microbiologically documented without bacteremia	6 (20)	3 (11)	-10 – 26	0.48
Clinically documented	3 (10)	6 (23)	-31 – 6	0.28

95% CI = 95% confidence interval.

that this difference was due to different intensities of chemotherapy. Although more patients in Group 1 presented HSV reactivation, the contribution of HSV per se to the severity of mucositis is difficult to assess, because of these baseline differences.

The duration of fever was slightly longer in Group 1; this could be due to the longer duration of neutropenia or to the higher proportion of patients with severe (grade 4) mucositis in this group. All patients who presented HSV reactivation had prolonged fever. This observation is in accordance with previous studies, which showed that patients with HSV reactivation have a more prolonged duration of fever during neutropenia [14]. Acyclovir reduces the duration of fever and prevents not only HSV reactivation but also Gram-positive bacteremia [15]. This is not unexpected, since HSV reactivation is associated with more severe mucositis and mucositis and is a risk factor for Gram-positive bacteremia in ASCT [16]. We did not observe any difference in the rates of Gram-positive bacteremias, but our study was not designed to demonstrate such differences.

Our study has many limitations, the most important being the non-randomized nature of allocation in the two arms. Non-randomized studies are more prone to result in significant imbalances that may considerably reduce the strength of the conclusions. Indeed, in our study, an unexpectedly higher proportion of patients in Group 1 had grade 4 baseline mucositis, making it difficult to interpret the results in terms of the prevention of severe mucositis between the two arms. Another limitation is that although the study had adequate power to achieve the primary endpoint, it was not designed to detect differences in the analysis of secondary events.

Despite these limitations, the results of our study have some important practical implications. The schedule of 125 mg/m^2 every six hours consumes three vials (250 mg) of acyclovir per day and the regimen of 60 mg/m^2 every six hours requires 1.5 vials per day (acyclovir is stable for 24 hours after reconstitution). If we consider that both groups had the same duration of acyclovir use (it was slightly lower in Group 2), a 50% reduction in the total cost with acyclovir would be achieved using the lower dose, without compromising the effectiveness of HSV prophylaxis. Considering that acyclovir accounts for almost 50% of the total cost of antimicrobial agents in our institution (data not shown), the cost reduction can be considerable.

In conclusion, acyclovir at a dose of 60 mg/m^2 every six hours seems to be as effective as a dose of 125 mg/m^2 every six hours in the prophylaxis of HSV reactivation in ASCT recipients during neutropenia. Prospective randomized studies will be needed to confirm our results.

References

1. Saral R., Burns W.H., Prentice H.G. Herpes virus infections: clinical manifestations and therapeutic strategies in immunocompromised patients. Clin Haematol **1984**;13:645-60.
2. Hann I.M., Prentice H.G., Blacklock H.A., et al. Acyclovir prophylaxis against herpes virus infections in severely immunocompromised patients: randomised double blind trial. Br Med J (Clin Res Ed) **1983**;287:384-8.
3. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant **2000**;6:659-713.
4. Saral R., Burns W.H., Laskin O.L., et al. Acyclovir prophylaxis of herpes-simplex-virus infections. N Engl J Med **1981**;305:63-7.
5. Gluckman E., Lotsberg J., Devergie A., et al. Prophylaxis of herpes infections after bone-marrow transplantation by oral acyclovir. Lancet **1983**;2:706-8.
6. Wade J.C., Newton B., Flournoy N., Meyers J.D. Oral acyclovir for prevention of herpes simplex virus reactivation after marrow transplantation. Ann Intern Med **1984**;100:823-8.
7. Angelopulos C., Gore S., Braine H.G., et al. Successful intravenous acyclovir (ACV) prophylaxis against Herpes Simples Virus (HSV) infection/reativation during Intensive chemotherapy of leukemia: 1000 courses (abstract H-143, page 205). Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, **1995**.
8. Arvin A.M., Prober C.G. Herpes Simples viruses. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of Clinical Microbiology. Washington, D.C.: American Society for Microbiology Press, **1995**;876-83.
9. Zirn J.R., Tompkins S.D., Huie C., Shea C.R. Rapid detection and distinction of cutaneous herpes virus infections by direct immunofluorescence. J Am Acad Dermatol **1995**;33:724-8.

10. Nucci M., Landau M., Silveira F., et al. Application of the IDSA guidelines for the use of antimicrobial agents in neutropenic patients: impact on reducing the use of glycopeptides. Infect Control Hosp Epidemiol **2001**;22:651-3.
11. Hughes W.T., Armstrong D., Bodey G.P., et al. 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. Clin Infect Dis **1997**;25:551-73.
12. Miller A.B., Hoogstraten B., Staquet M., Winkler A. Reporting results of cancer treatment. Cancer **1981**;47:207-14.
13. Beyer J., Schwella N., Zingsem J., et al. Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: a randomized comparison. J Clin Oncol **1995**;13:1328-35.
14. Baglin T.P., Gray J.J., Marcus R.E., Wreghitt T.G. Antibiotic resistant fever associated with herpes simplex virus infection in neutropenic patients with haematological malignancy. J Clin Pathol **1989**;42:1255-8.
15. Lonnqvist B., Palmblad J., Ljungman P., et al. Oral acyclovir as prophylaxis for bacterial infections during induction therapy for acute leukaemia in adults. The Leukemia Group of Middle Sweden. Support Care Cancer **1993**;1:139-44.
16. Cordonnier C., Buzyn A., Leverger G., et al. Epidemiology and risk factors for Gram-positive coccal infections in neutropenia: toward a more targeted antibiotic strategy. Clin Infect Dis **2003**;36:149-58.