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A double-blind randomized placebo-controlled study of oral glutamine in the prevention of mucositis in children undergoing hematopoietic stem cell transplantation: a pediatric blood and marrow transplant consortium study

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Summary:

Severe mucositis is a common cause of morbidity in hematopoietic stem cell transplant (HSCT) recipients. Glutamine has been shown to reduce mucositis in children receiving chemotherapy. Patients were randomized in a double-blind manner to receive glutamine or glycine at a dose of 2 g/m^2/dose (maximum dose 4 g) twice daily until 28 days post transplant or discharge if sooner. Mucositis was graded by use of a modified Walsh scale. A total of 120 children were evaluable: 57 children received glutamine and 63 received glycine. The mean mucositis score was 3.0 ± 0.3 *vs* 3.9 ± 0.4 ($P = 0.07$) in the glutamine and glycine groups, respectively. The glutamine group demonstrated a reduction in mean number of days of intravenous narcotics use (12.1 ± 1.5 *vs* 19.3 ± 2.8 in the glycine group, $P = 0.03$) and total parenteral nutrition (17.3 ± 1.7 *vs* 27.3 ± 3.6 in glycine group, $P = 0.01$). There was no statistically significant difference in toxicity between the two groups. Glutamine appears to be safe and beneficial in reducing the severity of mucositis. Strong consideration should be given to include oral glutamine supplementation as a routine part of supportive care of SCT patients.

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Mucositis remains a significant complication of hematopoietic stem cell transplantation (HSCT) seen in over 80% of transplant recipients,[1,2] and 42% report it to be the most debilitating side effect of the procedure.[3] The incidence and severity of mucositis is variable and is dependent on the type of conditioning regimen used, the presence of infections such as herpes simplex virus (HSV), the severity of graft-versus-host disease (GVHD) and the use of methotrexate and other drugs for GVHD prophylaxis and treatment.[4]

Management of mucositis has been primarily supportive with the use of intravenous narcotics and total parenteral nutrition (TPN).[5] A variety of interventions have been used to reduce the incidence of mucositis including topical agents such as vancomycin paste,[6] sucralfate,[7–9] G-CSF,[10] GM-CSF,[11,12] and lidocaine.[13,14] Systemic agents such as amifostine[15,16] and recombinant human epidermal growth factor[17] have also studied in HSCT patients. These interventions have been shown to have variable efficacy in the prevention and treatment of mucositis, and may have potential contraindications to their use in HSCT recipients.

Although considered a nonessential amino acid, multiple studies have shown that glutamine is an essential amino acid for enterocytes, and is depleted during damage to enteric mucosa.[18] Prior studies have suggested that supplementation with glutamine may prevent chemotherapy-related mucositis, although randomized studies in adult HSCT recipients have shown mixed results.[2] A previous study in children with cancer who received conventional chemotherapy demonstrated that glutamine use was associated with a decrease in the severity of mucositis.[19] We therefore conducted a double-blind placebo-controlled randomized study to determine if glutamine was efficacious in the amelioration of mucositis in children undergoing stem cell transplantation.

Materials and methods

Patient population

All children and adolescents who were candidates for allogeneic or autologous HSCT from any stem cell source (bone marrow, peripheral blood stem cells or placental blood) at participating centers of the Pediatric Blood and Marrow Transplant Consortium (PBMTC) between April 1998 and December 2002 were eligible for study enrollment.

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These children were transplanted after undergoing a variety of institutional and cooperative group regimens including those containing total body irradiation (TBI). The protocol was initially designed to enroll a total of 120 evaluable patients, but an additional 10 patients were offered enrollment to replace subjects deemed ineligible. The study was approved by the institutional review boards of the participating institutions and written informed consent was obtained from all subjects.

Patient eligibility

Patients were eligible for enrollment if they were undergoing allogeneic or autologous stem cell transplant from any stem cell source (including bone marrow, umbilical cord blood, or peripheral blood stem cells) at participating institutions, were younger than 21 years of age and their planned conditioning regimen was associated with at least a documented 50% risk of grade III or IV mucositis by NCI criteria.[20] Subjects were ineligible if they were to receive vancomycin paste or nonabsorbable antibiotics, were to receive glutamine-supplemented TPN, had a history of veno-occlusive disease, or were undergoing a second HSCT.

Study execution

Pediatric patients scheduled for stem cell transplantation who fulfilled all of the inclusion criteria were offered entry into the study. Patients were enrolled 7 days prior to admission for BMT and randomized in a double blind, randomized manner between glutamine and glycine using a random permutation table at a central pharmacy (Children's Medical Center Dallas). The drug was then shipped to the transplanting center. Glycine, an inert amino acid, was chosen as a placebo for this study. Patients were stratified based upon whether the conditioning regimen contained TBI. Oral glutamine at a dose of 2 g/m^2/dose (maximum dose 4 g) or oral glycine at a dose of 2 g/m^2/dose (maximum dose 4 g) was administered in a solution of 500 mg/ml twice daily beginning on the day of admission for HSCT. The drug was dissolved in water or other liquid at the local institution. The solution had the consistency of a thickened liquid. The study drug was administered until 28 days post transplant or until the day of discharge, whichever occurred first. The drug dose was not repeated if vomiting occurred. Other mouth care was administered per local institutional protocol (excluding vancomycin paste and nonabsorbable antibiotics).

Patients were examined daily and mucositis graded by a modified Walsh scale (Table 1).[21] It was recommended that a single observer be selected at each institution to assign the mucositis score. In general, nursing staff performed the evaluation, and filled out the daily mucositis-scoring sheet. The mean and highest mucositis scores were then calculated for analysis. The decision to initiate narcotic therapy or TPN was based upon institutional protocol. The number of days and severity of mucositis, gastrointestinal (GI) symptoms, fever, TPN, antibiotic use, episodes of bacteremia, and hospital days were collected. Patients were followed until discharge or until 28 days post transplant, whichever occurred first. Patients were withdrawn from the study if they refused to take the drug, developed a serum ammonia $>90\,\mu$mol/l, or had any change in mental status. A Data Safety and Monitoring Committee composed of members of the Pediatric Blood and Marrow Transplant Consortium, who were not investigators, provided oversight of the study. The protocol was evaluated every 6 months for violation of stopping rules if excess toxicity was detected in the group of patients receiving glutamine.

Table 1 Criteria for mucositis scoring (based on Walsh[21])

Category	*Signs*	*Score*
Lips	Normal, smooth, pink, moist	0
	Dry or cracked	1
	Dry, cracked with blisters, and/or bleeding	2
Tongue (lateral borders, dorsum and ventralsurfaces)	Pink, moist, papillae present	0
	Coated or, loss of papillae, (atrophic) or edema, no ulcers	1
	Erythema with ulcers and/or cracked, bleeding	2
Buccal and labial mucosa (left and right sides) and/or bleeding	Pink and moist	0
	Erythema or pseudomembrane, or edema, no ulcers	1
	Erythema and ulcerations, and/or bleeding	2
Hard/soft palate	Pink and moist	0
	Erythema or pseudomembrane, or edema, no ulcers	1
	Erythema and ulcerations, and/or bleeding	2
Gingiva	Pink and stippled	0
	Edematous and/or erythema	1
	Edematous, erythema; with bleeding pseudomembrane, or ulceration	2
Teeth	Clean, no debris	0
	Plaque or debris localized	1
	Generalized debris along gingival	2
Saliva production	Moist mouth	0
	Dry but saliva present	1
	Very dry, parched dull teeth	2
Pt/nurse assessment	No discomfort	0
	Some complaint of pain	1
	Significant complaint of pain	2

Statistical analysis

Data were entered into a computerized database (Microsoft Access 2003) and were analyzed by either Fisher's exact test or a χ^2 test for categorical data and the Mann–Whitney *U*-test for noncategorical data. The mean mucositis score was the primary end point analyzed and power calculations were made for this parameter. Data analysis was performed using SPSS version 10 (SPSS, Inc.). As initially designed,

the study was powered so that if 60 evaluable patients were in each arm the study would have the ability to detect a statistical difference with a power of 80% and a $P=0.05$ a 20% decrease in the mean mucositis scores of the subjects between the two groups. Secondary outcomes analyzed included antibiotic days, episodes of bacteremia, hospital days, and febrile illness and mortality during the transplant. A $P\leqslant 0.05$ was considered statistically significant.

Results

Patient population

A total of 130 eligible patients were enrolled between April 25, 1998 and December 2, 2002. The study initially enrolled 120 patients of whom 10 were subsequently found to be ineligible for the following reasons: eight refused to take a single dose of the drug, one was enrolled but did not undergo transplant, and one was unable to receive drug due to an inability to ship the drug due to the terrorist attack on 9/11/01. After consultation with the DSMB, it was decided to enroll an additional 10 patients to complete enrollment. The 120 eligible patients are described in the rest of this report. Of these patients, 57 received glutamine and 63 received glycine.

The characteristics of the 120 patients are summarized in Table 2. There was no difference in the age, gender, or underlying diagnoses of the patients. There was a statistically significant difference in the number of patients who were known to be positive for HSV at the time of transplant in the placebo group *vs* the glutamine group ($P=0.04$), with more patients in the glycine group being positive for HSV as compared to the glutamine group. All children who were HSV-positive prior to transplant received either oral or intravenous acyclovir for prophylaxis during their transplant.

Transplant characteristics are summarized in Table 3. There was no difference in the number of patients who received TBI or methotrexate as a part of their GVHD prophylaxis. A similar number of patients underwent autologous and allogeneic transplants ($P=0.8$), and there was no difference in stem cell sources. There was no difference in the mean time to engraftment between the two groups ($P=0.6$).

Patient outcomes

The results of glutamine administration are summarized in Table 4. Glutamine was as well tolerated and appeared as palatable as glycine in this patient cohort. Patients complied with 76.6 ± 4.2 *vs* 74.2 ± 4.0% of the doses in the glutamine and glycine groups, respectively ($P=0.8$). A total of 100 mucositis scoring sheets were available for analysis, 52 (91%) in-patients who received glutamine and 48 (76%) in-patients who received glycine. There was a trend toward a reduction in the average mucositis score ($P=0.07$), although there was no difference in the maximum mucositis score ($P=0.7$). There was a statistically significant decrease in the median number of days of morphine use from 19.3 ± 2.8 in the glycine group to 12.1 ± 1.5 in the glutamine group ($P=0.01$), and a reduction in the median number of days of TPN use from 27.3 ± 3.6 in glycine group to 17.3 ± 1.7 in the glutamine ($P=0.02$).

We also analyzed the incidence of the toxicities associated with glutamine use described in previous studies. This analysis is summarized in Table 5. Although the study is not powered to detect small differences in adverse reactions between the two groups, there appeared to be no excess toxicity related to the use of glutamine.

As expected, glutamine did not have any effect on other complications of HSCT. There was no statistically sig-

Table 2 Characteristics of 120 transplant recipients who were randomized to receive glutamine or glycine

	Glutamine	*Glycine*	*P-value*
Age (years)	8.9 ± 1.0	10.5 ± 0.6	0.2
Sex			
Male	37 (65%)	36 (57%)	0.4
Female	20 (35%)	27 (43%)	
Diagnosis			
Leukemia	30 (53%)	32 (51%)	0.8
Lymphoma	3 (5%)	5 (8%)	0.6
Neuroblastoma	12 (21%)	10 (16%)	0.8
Other solid tumors	7 (12%)	11 (17%)	0.6
Nonmalignancy	5 (9%)	5 (8%)	0.9
HSV positive	12 (21%)	24 (38%)	0.04

Table 3 Characteristics of 120 transplants randomized to receive glutamine or glycine

	Glutamine	*Glycine*	*P-value*
Total body irradiation	24 (42%)	26 (41%)	0.9
Methotrexate as GVHD prophylaxis	16 (28%)	19 (30%)	0.8
Autologous (total)	25 (44%)	29 (46%)	0.8
Allogeneic (total)	32 (56%)	34 (54%)	
Stem cell source			
Bone marrow	29 (51%)	31 (49%)	1.0
Peripheral blood stem cells	23 (40%)	21 (33%)	0.5
Umbilical cord blood	5 (9%)	11 (18%)	0.2
Time to engraftment (days)	15.5 ± 1.5	14.5 ± 0.8	0.6

Table 4 Results of glutamine supplementation in 120 children undergoing hematopoietic stem cell transplantation

	Glutamine	*Glycine*	*P-value*
Percentage of doses taken	76.6 ± 4.2%	74.2 ± 4.0%	0.8
Average mucositis score	3.0 ± 0.3	3.9 ± 04	0.07
Highest mucositis score	7.2 ± 0.6	7.5 ± 0.6	0.7
Days of intravenous narcotic use	12.1 ± 1.5	19.3 ± 2.8	0.03
Days of TPN use	17.3 ± 1.7	27.3 ± 3.6	0.01
Episodes of patients who developed at least one episode of bacteremia	23 (40%)	26 (41%)	0.9
Hospital days	31.6 ± 2.1	34.0 ± 2.1	0.4

Table 5 Potential toxicities associated with the administration of glutamine in 120 children undergoing hematopoietic stem cell transplantation

	Glutamine	*Glycine*	*P-value*
Renal failure	4 (8%)	2 (3%)	0.4
Veno-occlusive disease	3 (6%)	4 (6%)	0.7
Mental status changes	2 (4%)	3 (5%)	1.0
Maximum serum ammonia	41.8±1.5	40.2±8.6	0.5
Mortality	6 (11%)	6 (9%)	0.8

nificant difference in the number of episodes of bacteremia ($P=0.9$) or the total number of hospital days ($P=0.4$) between the two groups. The day 100 mortality was similar between the two groups ($P=0.7$).

Discussion

Glutamine is a nonessential amino acid, which accounts for 60% of the amino-acid content of muscle.[22] In humans, glutamine plays a role in interorgan nitrogen transport, renal ammoniagenesis and acid–base balance, hepatic glutathione production, preservation of mucosal structure, and function in the intestine; and acts as a required substrate for proliferation of lymphocytes and macrophages.[23,24] During injury or stress, intracellular glutamine concentration falls by 40–50% (approximately 14 g of free glutamine) in uncomplicated cases[25,26] and does not return to normal levels for as long as 30 days after injury.[27,28] The extent of glutamine depletion has been correlated with an increased incidence of mortality.[27,28] Animal studies have shown that the GI mucosal breakdown produced by radiation and chemotherapy can be prevented by enteral or parenteral administration of glutamine. Klimberg *et al*[29] demonstrated that rats fed a diet poor in glutamine develop edema and ulceration of their GI tract with loss of villi. Rats given TPN without glutamine have impaired GI immune function, as measured by increased translocation of bacteria to mesenteric nodes and decreased biliary immunoglobulin A secretion. The addition of 2% glutamine to TPN reverses this immune dysfunction.[30]

Intravenous glutamine supplementation, usually added to standard TPN solution has been studied in adult stem cell transplant patients, although a benefit has not been seen in all studies.[31–42] Ziegler *et al*[42] randomized adult BMT patients to receive glutamine-supplemented or standard glutamine-free parenteral nutrition. They demonstrated a significant decrease in the incidence of clinical infection, positive cultures, hospital days, and improvement in nitrogen balance in the patients who received glutamine supplements. Schloerb *et al*[36] also studied stem cell transplant patients randomized to receive glutamine-supplemented TPN. The length of hospitalization was 5.8 days less in the glutamine-supplemented group. However, there was no difference in incidence of positive blood cultures, clinical infection and mortality in the two groups. Based on a mean reduction in hospitalization of 5.8 days, the estimated cost savings for the 30 stem cell transplant patients who received intravenous glutamine supplements per year totaled approximately $180 000. Providing glutamine-supplemented parenteral nutrition in the Ziegler *et al*[43] study resulted in savings of $1970/patient, or $330 000 for an equivalent 30 patients annually. Studies of oral glutamine, administered either alone or as a part of enteral enriched formulas have shown variable results.[19,29,44–49]

In this study, although there appeared to be a statistical trend toward a reduction in mucositis as subjectively measured by a clinical scoring scale, there was a statistically significant reduction in the number of days of morphine and TPN use, which are objective indicators of a decrease in the severity of mucositis. Unfortunately, it is not possible to know if a statistically significant difference between the two groups would have been detected if all of the mucositis scoring had been available for analysis. Given the relative expense of total parental nutrition, significant cost savings are associated with the use of glutamine in this group of stem cell transplant recipients. Although the use of glutamine has been associated with enhanced immune recovery,[34,50] there was no reduction in the number of infections in this group of HSCT recipients.

In this study, there were more children in the placebo group who were positive by serology for HSV prior to transplant. HSV is known to be a risk factor for the development of mucositis and may have influenced the results of the study. However, all patients received HSV prophylaxis, and no patients developed clinical HSV infection. Unfortunately, we had an inadequate number of patients to stratify for all known risk factors, and chose to stratify for the administration of irradiation since this is known to be a significant risk factor for mucositis in a previous PBMTC study.[6]

Based on these data, we conclude that glutamine appears to be safe and beneficial in reducing the severity of mucositis as demonstrated by a reduction in the use of intravenous narcotics and TPN. The lower incidence of HSV positive children in the glutamine group may have confounded the results, although all patients who were HSV-positive prior to transplant received acyclovir prophylaxis. Strong consideration should be given to including oral glutamine supplementation as a routine part of supportive care of SCT patients. Future studies might explore the addition of glutamine to palifermin or Traumeel® in an attempt to further reduce mucositis and its associated morbidities.

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