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Effect of low-dose oral glutamine on painful stomatitis during bone marrow transplantation

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Summary:

Painful oral mucositis is a common complication after bone marrow transplantation (BMT). Glutamine is a nutrient for rapidly dividing cells and the major energy source for intestinal epithelium. This study tested whether an oral glutamine preparation could decrease the severity of oral mucositis in patients undergoing BMT. Glutamine or a placebo (glycine) were administered from admission until day +28 in 193 BMT patients in a randomized, double-blind, placebo-controlled study at a dose of 1.0 g amino acid/m^2/dose swish and swallow four times a day. In autologous BMT patients (n = 87) glutamine was associated with significantly less mouth pain by self report and by opiate use (5.0 ± 6.2 days of morphine for glutamine *vs* 10.3 ± 9.8 days for placebo; P = 0.005). Matched sibling BMT patients had no effect by self report and an increased duration of opiate use (23.2 ± 5.7 days for glutamine *vs* 16.3 ± 8.3 days for placebo) (P = 0.002). However, day 28 survival of allogeneic patients was improved by glutamine. No significant differences in TPN use, rate of relapse or progression of malignancy, parenteral antibiotic use, acute or chronic GVHD, or days of hospitalization were observed in either autologous or allogeneic recipients. No toxicity of glutamine was observed. We conclude that oral glutamine can decrease the severity and duration of oropharyngeal mucositis in autologous BMT patients but not in allogeneic BMT patients, possibly due to interaction with methotrexate.

Keywords: mucositis; stomatitis; supportive care; nutrition; epithelium; chemotherapy

Many of the major adverse effects of BMT are related to disruption of mucosal integrity from high-dose chemotherapy and/or radiation-induced damage of oral and intestinal epithelium. Preclinical studies have demonstrated less damage to rat intestinal epithelium if high doses of enteral glutamine were included in the diet during and after 1000 cGy of whole abdominal radiation.[1–3] Glutamine is the most abundant amino acid in the blood, but is generally absent from total parenteral nutrition (TPN) for solubility and stability reasons.[4–7] The intestine utilizes glutamine as its primary energy source, and glutamine has been shown to be an essential dietary component for support and maintenance of intestinal growth and function.[8–10] Supplementation of TPN with glutamine has been shown to decrease villous atrophy associated with exclusive feeding via TPN.[11,12] Nutritionally depleted patients have been shown to have decreased plasma glutamine and mucosal glutamine.[13] Recent studies have also demonstrated a survival advantage of TPN supplementation with glutamine in critically ill patients.[14]

Since no information was available concerning the effect of oral glutamine on the squamous epithelium of the oropharynx after chemotherapy and/or radiation, we conducted an earlier pilot study using oral glutamine 4 g/m^2 twice a day in patients previously experiencing stomatitis. This study demonstrated the substantial benefit of low-dose oral glutamine on both duration and severity of stomatitis after conventional-dose intensive chemotherapy.[15] Since the prevalence of severe oral mucositis after BMT is high, we evaluated an oral mouth rinse of a glutamine suspension or placebo in BMT patients in a randomized, double-blind, placebo-controlled study. Results indicate that oral glutamine was effective in reducing opiate use for mouth pain in autologous BMT patients but not in allogeneic BMT patients.

Patients and methods

Patients

The protocol was approved by the University of Minnesota Institutional Review Board. Informed consent for the study was obtained from all patients. There were no age or disease-type exclusions and all consecutive patients undergoing BMT at the University of Minnesota were eligible. Due to anticipated differences in severity of mucositis as well as graft-versus-host disease (GVHD), patients were prospectively stratified by type of transplant (autologous, matched sibling donor, or unrelated donor) and randomized to 'Mouth Care Study Suspension A' or 'Mouth Care Study Suspension B'. Study suspension was provided to the BMT patients (1 g/m^2 swish and swallow four times a day; 2 cc/m^2 per dose) during preparative chemotherapy and radiation, and then continued until 28 days after marrow

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Table 1 Patient characteristics

	Glutamine	*Placebo*
Type of BMT		
Autologous	45	42
Matched sibling donor	27	28
Unrelated donor	26	25
Total	98	95
Age		
0–9	19	21
10–19	15	17
20–29	7	10
30–39	24	17
40–49	21	20
50+	12	10
Mean age (range)	29 (1–62)	27 (1–62)
Gender		
Male	52	59
Female	46	36

infusion. This dose was chosen because (1) it is the same daily dose of oral glutamine used in patients who benefited in a pilot study of cancer patients receiving chemotherapy associated with prior mucositis;[15] and (2) pragmatic concerns for the comfort of subjects who would swallow a suspension four times a day for 5 weeks while participating in this study during preparative chemotherapy and BMT. In these patients, who are often quite nauseous, it was felt that 4 ml would be an acceptable unit dose suspension volume.

Of 195 consecutive BMT patients, all but two elected to participate in this study. Table 1 summarizes the characteristics of the randomized and stratified cohorts. Glutamine and placebo groups were comparable with respect to type of donor ($P = 0.95$), age distribution ($P = 0.83$), gender ($P = 0.20$) and diagnosis ($P = 0.95$). Table 2 details the similar indications for BMT in the glutamine and placebo groups. Table 3 describes the prevalence of two variables known to augment stomatitis, radiation-containing preparative regimens and the use of methotrexate for GVHD prophylaxis. Radiation during the preparative regimen was less common in autologous BMT patients (54/87; 62%) than matched sibling BMT patients (48/55; 87%), or unrelated donor patients (42/51; 82%). None of the 87 autologous patients received methotrexate during the study; 47/55 (85%) of the matched sibling donor patients received methotrexate as a component of GVHD prophylaxis, as did 42/51 (82%) of the unrelated donor patients. Thus, randomization was successful and achieved similar glutamine and placebo groups.

Table 2 Indications for BMT

Disease category[a]	*Glutamine*	*Placebo*
Solid tumor	29	33
ALL	13	12
ANLL/MDS	18	19
CML	24	20
Aplastic anemia	4	4
Inherited diseases	10	7

[a]Solid tumors included lymphoma, breast cancer, multiple myeloma, neuroblastoma, rhabdomyosarcoma, Wilms tumor and glioblastoma.
ALL = acute lymphoblastic leukemia; ANLL/MDS = acute non-lymphocytic leukemia and myelodysplastic syndrome; CML = chronic myelogenous leukemia.
Inherited diseases included severe combined immunodeficiency, adrenal leukodystrophy, Hurler's syndrome, Wiskott–Aldrich syndrome.

Table 3 Additional possible variables affecting stomatitis severity: radiation in BMT preparative regimen and use of methotrexate for GVHD prophylaxis

	Glutamine	*Placebo*
Radiation		
Autologous	30	24
Matched sibling donor	23	25
Unrelated donor	19	23
Methotrexate		
Autologous	0	0
Matched sibling donor	22	25
Unrelated donor	25	21

Amino acid suspension information

Glutamine is relatively insoluble; therefore suspensions are necessary to achieve concentrated doses. Glutamine and glycine (placebo) suspensions were prepared at a concentration of 500 mg/ml weekly by the University of Minnesota Investigational Pharmacy. Crystalline amino acids suitable for human use were purchased from Ajinomoto USA (Teaneck, NJ, USA) (FDA IND No. 36,978). The vehicle was two parts Ora-Sweet SF vehicle, one part water, and one part Ora Plus (Paddock Laboratories, Minneapolis MN, USA). The color, odor, texture and taste of the glutamine and glycine suspensions were virtually identical. Neither nurses, physicians, nor patients receiving the amino acid suspension knew whether it was glutamine or placebo.

Data collection and statistical analysis

The University of Minnesota BMT Database prospectively collected data regarding: radiation and methotrexate use during BMT, day of discharge from the hospital, relapse or progressive disease, bacterial and viral infections, GVHD status and survival. Two clinical pharmacists on the BMT unit (who were blinded to the study drug) prospectively collected data daily on opiate requirement for amelioration of painful stomatitis, TPN use, and gram negative and/or gram positive parenteral antibiotic administration. Due to the severity of pain and the prevalence of nausea and thrombocytopenia, parenteral opiates were used for the amelioration of mucositis pain. Data collected by BMT pharmacists was available for all patients of each cohort. Patients completed a self-report calendar assigning a number to indicate severity of mouth pain and its effect on oral intake (0, none; 1, mild; 2, soft foods; 3, liquids only; 4, unable to swallow liquids) and the presence or absence of

stomatitis (N, no pain; M, sore mouth or sores present). Self-report calendars were 100% complete in 42–64% of patients of each treatment group. Self-report data collection was incomplete for a number of reasons including forgetting, being too ill to mark a score down, or being discharged before day 28. No significant difference in completeness of data collection was seen between glutamine and placebo data sets.

Opiate use was chosen as an objective measure of mucositis severity due to its quantitative nature and freedom from inter-observer bias. Frequency and duration of opiate use were analyzed as a measure of mucositis severity using both days of opiate administration and the proportion of days of opiate use per days at risk while on amino acid suspension. Self-reports of mouth pain and mouth sores were analyzed using the sum of daily mucositis scores as an index of severity. Presence of mouth sores on days 0–7, 0–14 and 0–28 was utilized as an index of duration of stomatitis among patients who returned complete calendars. Differences between means were calculated using a two-tailed *t*-test. Relapse and survival analysis used Kaplan–Meier estimates and comparisons between groups used the log rank test of significance.

Results

Severity, presence and duration of stomatitis by self-report

Table 4 details the severity of stomatitis in autologous, matched sibling donor, and unrelated donor BMT patients as indicated by the sum of daily oral mucositis scores on a self-report calendar in this randomized and double-blinded trial. Less mouth pain and less difficulty in eating was seen after low-dose oral glutamine in autologous BMT patients ($P = 0.05$). The presence and duration of painful mouth sores after BMT as indicated by the percent of patients with stomatitis at days 3, 7 and 14 are illustrated in Figure 1. As expected, allogeneic BMT patients had a longer duration of stomatitis and more severe oral mucositis than autologous BMT patients.

Table 4 Severity of oral mucositis after BMT (self-report)

Days after BMT	*Glutamine*[a]	*Placebo*[a]	*P value*[b]
Autologous			
0–7	10.7 ± 12.9	21.7 ± 24.1	0.05
0–14	14.8 ± 18.5	37.4 ± 41.0	0.02
0–28	14.8 ± 18.5	41.4 ± 51.9	0.04
Matched sibling			
0–7	29.5 ± 21.2	33.7 ± 25.6	0.64
0–14	74.1 ± 28.0	71.9 ± 49.1	0.88
0–28	142.0 ± 50.0	137.0 ± 93.0	0.84
Unrelated donor			
0–7	25.6 ± 18.3	27.5 ± 11.3	0.75
0–14	62.0 ± 38.6	74.6 ± 19.8	0.32
0–28	95.9 ± 58.5	129.0 ± 55.9	0.15

[a]Mean of sum of scores on self-report calendars ± s.d.
[b]Welch *t*-test *P* value comparing glutamine *vs* glycine placebo.

Opiates for painful stomatitis

Opiate use for stomatitis was quite common after BMT and is detailed in Table 5. Among autologous BMT patients, the number of patients requiring no morphine during days −10 to +28 was significantly greater in the glutamine group compared to the placebo group (53 *vs* 31%, $P = 0.04$), while no statistically significant difference in opiate use was observed in the matched sibling donor or unrelated donor patients. The duration of opiate administration was also significantly less in the autologous BMT patients provided glutamine suspension compared to placebo (5.0 ± 6.2 days *vs* 10.3 ± 9.8 days, $P = 0.005$). Interestingly, glutamine was associated with an increased duration of morphine use in BMT patients with grafts from matched sibling donors (23.2 ± 5.7 days for glutamine *vs* 16.3 ± 8.3 days for the placebo, $P = 0.002$), while no difference was seen in the duration of morphine use in unrelated donor BMT patients (Table 5).

Because early deaths could be associated with fewer days of morphine use, opiate use was also calculated as a proportion of days at risk during days 0 to +28 after BMT (Table 6). Opiate use as a proportion of days at risk during the preparative regimen and the first 4 weeks after BMT

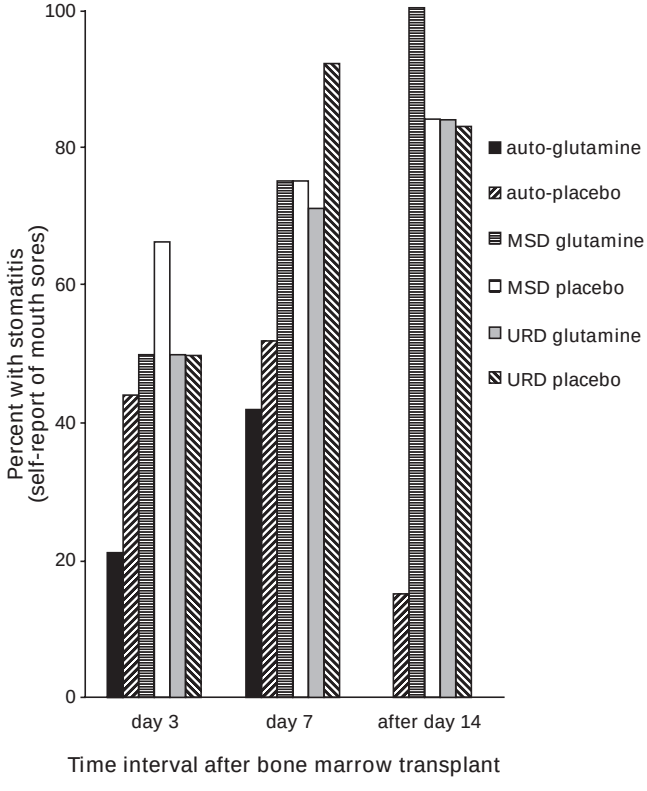

Figure 1 Frequency of stomatitis (all grades >0) after bone marrow transplant as a function of time. The percent of patients with mouth sores of any severity by self-report in the autologous, matched sibling donor (MSD), and unrelated donor (URD) cohorts treated with glutamine or placebo are shown as indicated.

Table 5 Opiate use during BMT for painful stomatitis

Type of BMT	*Glutamine*	*Placebo*	*P value*
No. of patients requiring no opiates days 0 to 28 (%)			
Autologous	24/45 (53)	13/42 (31)	0.04[a]
Matched sibling donor	0/27 (0)	3/28 (11)	0.08
Unrelated donor	3/26 (12)	1/25 (4)	0.32
Days of opiate use ± s.d.			
Autologous	5.0 ± 6.2	10.3 ± 9.8	0.005[b]
Matched sibling donor	23.2 ± 5.7	16.3 ± 9.3	0.002
Unrelated donor	20.7 ± 9.9	20.4 ± 8.4	0.91

[a]Chi-square test *P* value comparing glutamine *vs* glycine placebo
[b]Student's *t*-test *P* value comparing glutamine *vs* glycine placebo.

Table 6 Opiate use as proportion of days 0 to 28

Type of BMT	*Glutamine*	*Placebo*	*P value*[a]
Autologous	0.21 ± 0.26	0.41 ± 0.38	0.02
Matched sibling donor	0.82 ± 0.20	0.62 ± 0.34	0.007
Unrelated donor	0.72 ± 0.33	0.74 ± 0.28	0.44

[a]Student's *t*-test *P* value comparing glutamine *vs* glycine placebo.
The number of days of opiate use/total number of observed days (day 0 to +28) are shown. Patients were censored at the time of death.

was also significantly less in autologous patients receiving the oral glutamine suspension ($P = 0.005$, Table 6). However, glutamine supplementation was also associated with increased morphine use in BMT patients receiving grafts from matched sibling donors ($P = 0.005$, Table 5). No differences in morphine use were seen comparing glutamine or placebo groups of BMT patients who received grafts from unrelated donors (Table 6).

Antibiotic and TPN use

As expected, nearly all patients during BMT developed fevers and received parenteral antibiotics. No meaningful differences were observed in days of TPN use, gram-negative antibiotic use, or gram-positive antibiotic use in the glutamine compared to placebo groups in autologous, matched sibling donor or unrelated donor patients.

Late outcomes: GVHD, survival, infections

No differences in ⩾ grade 2 acute graft-versus-host disease, the median time to ⩾ grade 2 acute graft-versus-host disease, or chronic (1 year) graft-versus-host disease were observed between the glutamine or placebo groups for either the matched sibling or unrelated donor patient groups ($P > 0.2$). There were no differences in numbers and types of bacterial or fungal infections in glutamine and placebo groups. There were seven viral infections before day 100 in the placebo group and none in the glutamine-treated group. The early (day 28) mortality was observed to be significantly different between the glutamine (0/98) and placebo (7/95) groups in favor of glutamine (Table 7, $P = 0.006$), however, this difference was no longer significant at day 100 (glutamine, 12/98 *vs* placebo, 18/95, $P = 0.07$).

Discussion

Severe stomatitis from regimen-related toxicity during BMT is perhaps the major source of discomfort associated with bone marrow transplantation. Both radiation-containing preparative regimens and the use of methotrexate for GVHD prophylaxis may independently contribute to this complication. Our institution previously studied the effects of chlorhexidine on mucositis and observed no benefit in either autologous or allogeneic BMT recipients.[16] Randomized studies using pentoxyfilline in the BMT setting also demonstrated no amelioration of mucositis or regimen-related toxicity.[17] One promising means of reducing chemotherapy-associated stomatitis has been the use of ice chips in the mouth for 30 min during 5-fluorouracil infusions.[18] Except for this maneuver, there are no reported effective agents that reduce oral mucositis.

Table 7 Percent survival

	Glutamine	*Placebo*	*P value*[a]
Day 28			
Autologous	100	97.6	0.30
Matched sibling donor	100	85.7	0.04
Unrelated donor	100	92.0	0.15
All patients	100	92.6	0.006
Day 100			
Autologous	90.8	95.2	0.44
Matched sibling donor	88.9	71.4	0.10
Unrelated donor	78.6	67.2	0.25
All patients	87.2	80.9	0.18

[a]Log-rank test *P* value comparing glutamine *vs* glycine placebo.

There are theoretical reasons why oral glutamine might be useful for chemotherapy and radiation therapy induced mucositis.[10] Our earlier nonrandomized study of oral glutamine in which patients served as their own controls suggested benefit,[15] and prompted this randomized trial in BMT patients. The dose of glutamine used in our study is somewhat greater than the average dietary intake (2–5 g/day) but within a range that one may achieve with a high protein diet containing gluten which is composed of 35% glutamine.[19] Since a 2-g oral dose of glutamine can significantly raise (~0.1 mm difference) blood glutamine levels for about 1 h,[20] effects seen with the dose used in our study may be related to either local or absorbed glutamine, however a beneficial effect of intravenous glutamine on stomatitis was not seen in other studies.[21] Since we observed less frequent and a shorter duration of mouth pain both by self-report and by opiate use in autologous patients receiving glutamine, we conclude that oral glutamine can ameliorate the duration of pain in the oropharynx associated with mucositis due to high-dose chemotherapy and radiation after autologous BMT.

Allogeneic BMT patients have more severe and prolonged oropharyngeal mucositis than patients with autologous grafts. In the current study, glutamine did not prevent or reduce the painful stomatitis in allogeneic BMT recipients. Although both radiation-containing preparative regimens and the use of methotrexate for GVHD prophylaxis may independently contribute to this stomatitis, glutamine and placebo groups were similar with respect to the use of these therapies. Methotrexate for GVHD prophylaxis was a major difference between the autologous and allogeneic cohorts (0 *vs* 84%). It has recently been demonstrated that glutamine can inhibit renal clearance of methotrexate, thereby possibly exposing the host to greater methotrexate concentrations and increased mucositis.[22] Thus, mucositis severity in the allogeneic host and the lack of response to oral glutamine in this study may be due in part to the additional tissue damage resulting from a methotrexate–glutamine interaction. This was not known when the study was designed, and future studies in allogeneic patients will need to take possible glutamine–methotrexate interactions into account. Finally, since glutamine is a critical nutrient for the immune system, it is possible that a local augmentation of GVHD could contribute to a worse stomatitis in allogeneic patients.

Since glutamine has previously been shown to be an energy source not only for rapidly dividing intestinal epithelial cells and lymphocytes,[1–3,23,24] but also tumor cells,[10,25,26] we chose an initial dose of glutamine that would mimic the effects of a high protein diet so as not to unduly increase the possibility of relapse or progression of malignancy. No adverse effects of glutamine were observed in this randomized double-blind study.

A suspension was used in order to achieve a high concentration of glutamine in an oral swish and swallow preparation. The oral route was chosen instead of the intravenous route in our study because of promising results in our earlier oral glutamine pilot study in chemotherapy patients[15] and preclinical oral glutamine studies in a rat methotrexate model. The latter showed superior survival after oral *vs* parenteral glutamine.[27]

Oral glutamine was also associated with improved survival at day 28 in our study. One possible explanation is that although stomatitis may be worse in matched sibling donor allogeneic patients receiving glutamine and methotrexate, the gut may be protected. Another possible mechanism of glutamine contributing towards better survival is improvement in the buffering capacity of the blood (ie increased plasma bicarbonate) and/or higher growth hormone levels and subsequent anabolism in response to low-dose oral glutamine.[20] Since critically ill patients have also been shown to have improved survival with glutamine supplemented TPN,[14] it is also possible that improved outcome could be related to absorbed glutamine and improved protein-energy metabolism and glutathione synthesis.

Different observations regarding the efficacy of oral glutamine[15,28] for amelioration of chemotherapy-associated mucositis could be related to different glutamine concentrations in the oral mucosa, as well as the route and schedule of administration. Because of poor solubility and biodistribution, only a suspension can achieve a high local concentration in the oropharynx. If glutamine is either dissolved[28] or given intravenously,[21,29] the concentration in the oral mucosa may be insufficient to significantly ameliorate mucositis and its associated pain.

It is also possible that benefits of oral and i.v. glutamine are different. High doses of i.v. glutamine (14–17 g/m^2/day, or approximately four times that used in our study), have been reported to significantly reduce the length of hospital stay, incidence of serious infections and improve nitrogen balance in autologous BMT patients.[21,29] However, no effect on oral mucositis was seen when i.v. glutamine was tested. The dose of oral glutamine chosen in our study may have predominantly local effects in the mouth, since no significant effect on antibiotic use or early discharge from hospital was seen.

Recent reports indicate that the oral mucosa may not contain enough glutaminase to utilize glutamine as a major energy source for oxidative metabolism.[30,31] Other mechanisms of glutamine action could be related to anabolic properties[2,6,9] or its analgesic and anti-inflammatory properties.[32] Studies in man have shown that low-dose oral glutamine significantly increases plasma bicarbonate, the major buffer of acid in the blood, as well as growth hormone.[20] Since glutamine is a critical nutrient for the immune system,[19] a trophic effect on the submucosal lymphoid system may also be an important mechanism of the observed beneficial effect. Finally, since glutamine acts in enterocytes with other factors that promote cellular proliferation and repair, including epidermal growth factor and insulin-like growth factor I,[33] similar mechanisms may be possible in oral epithelium. It has been demonstrated that glutamine can activate extracellular signal-related kinases and nuclear kinases in enterocytes. It is possible that similar or related mechanisms may alter gene regulation in the oral epithelium to promote proliferation and repair.

We conclude that oral glutamine supplementation is a simple, safe and effective way to decrease the severity of oropharyngeal mucositis in autologous BMT, an important cause of morbidity associated with this treatment. Given the current morbidity associated with marrow transplantation, the wide safety margin of oral glutamine,[15,34,35] and

the known physiologic production of glutamine by muscle and adipose tissue,[36] the risk/benefit of oral glutamine as administered in this study appears highly favorable.

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