Oral glutamine to prevent chemotherapy induced stomatitis: A pilot study

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Mucositis is a common toxicity of cancer chemotherapy. Glutamine appears to be the major energy source for intestinal epithelium, and animal studies have suggested that dietary supplementation with glutamine may protect the gut from both radiation and chemotherapy. Patients experiencing stomatitis after a course of chemotherapy were offered the opportunity to enter the current study if no clinical parameters precluded receiving the same chemotherapy doses during the next course of treatment. Patients received the same chemotherapy regimen as during the previous treatment but in addition received a suspension of L-glutamine, 4 gm swish and swallow twice a day, from day 1 of chemotherapy for 28 days or for 4 days past the resolution of any post-chemotherapy mucositis. Twelve patients receiving doxorubicin, 1 receiving etoposide, and 1 receiving ifosfamide, etoposide, and carboplatinum were entered into the study. The maximum grade (CALGB criteria) of mucositis decreased in 12 of 14 patients with glutamine supplementation (median score 2A vs 0.5, $p < 0.001$). Similarly, after glutamine supplementation, the total number of days of mucositis was decreased in 13 of 14 patients (2.7 ± 0.8 (mean ± SEM) vs 9.9 ± 1.1, $p \geq 0.001$). Thirteen of the 14 patients felt that the mucositis was less severe with the addition of glutamine. No change in the nadir neutrophil count was noted with glutamine, and no toxicity of glutamine was observed. We conclude that oral supplementation with glutamine can significantly decrease the severity of chemotherapy-induced stomatitis, an important cause of morbidity in the treatment of patients with cancer. Glutamine supplementation in patients receiving therapy for cancer warrants further study. (J LAB CLIN MED 1996; 127:223-8)

Mucositis is a common limiting toxicity of cancer chemotherapy. The mechanism of chemotherapy-induced mucositis may be multifactorial. Presumably chemotherapy damages the rapidly dividing immature intestinal crypt cells in the gut and more superficial immature mucosal cells

in the oropharynx.[1-4] Although perhaps more the result rather than the cause of mucositis, the phenomenon of bacterial translocation across a malfunctioning gut epithelium may also play a role in the gut-related toxicity of chemotherapy and radiotherapy.[3,5,6]

Glutamine is the most abundant amino acid in the blood and in the total body amino acid pool, and there has recently been much interest in its role in nutrition (reviewed in references 5 and 7 through 10). Glutamine is a "non-essential" amino acid in that it can be synthesized by most tissues. However, although the metabolism of some tissues such as skeletal muscle and brain yield a net synthesis and export of glutamine, cells of other tissues utilize glutamine as a nitrogen source and also as an energy source.[7] Glutamine appears to be the major energy source for intestinal epithelium.[7] In addition to be-

ing a primary fuel for gut enterocytes, glutamine may be essential for gut epithelium (reviewed in references 1 and 7). For example, glutamine supplementation of total parenteral hyperalimentation decreases the villous atrophy associated with exclusive feeding via TPN.[11]

Animal studies have suggested that supplementation of an elemental diet with glutamine may protect the gut from radiation and some chemotherapeutic agents (reviewed in references 2 and 4). When rats were treated with elemental diets enriched in either glutamine or glycine before abdominal radiotherapy, rats in the glutamine group had a more normal mucosal structure and had a better survival rate than rats in the glycine-enriched group.[12] Studies in rats treated with methotrexate demonstrated that glutamine supplementation of an elemental diet resulted in less weight loss, increased mucosal weight of the jejunum and colon, longer survival, lower mortality rate, and a lower incidence of bacteremia.[2,3]

We hypothesized that the recovery of cells from damage (either chemotherapy-induced or radiation-induced) would be enhanced by providing the cells with an optimal nutrient/energy source. Although it is known that the lower gastrointestinal tract obviously absorbs nutrients and can utilize them as they pass through the absorptive cells, it is not known to what extent cells lining the mouth and esophagus can utilize luminal nutrients. We hypothesized that the local administration of relatively high concentrations of glutamine by "swish and swallow" might result in some local absorption of this nutrient/energy source by the cells lining the mouth and esophagus. We reasoned that if this occurred, the cells could likely utilize these nutrients directly and that this would prevent damage and assist recovery from chemotherapy or radiotherapy. If such local absorption were successful, nutrients could be provided to the cells in question while at the same time adding a minimal caloric load to the patient, thereby eliminating the risk of "feeding the tumor." Rather than test the hypothesis that these cells could indeed absorb and utilize amino acids presented in this way, we tested the more relevant hypothesis—that oral glutamine could prevent or minimize stomatitis—by conducting a study of oral glutamine supplementation in patients experiencing stomatitis after chemotherapy. In this study the glutamine provided about 30 kcal/day of energy, a very small percentage of daily caloric intake.

METHODS

Patients experiencing stomatitis after a course of chemotherapy were offered the opportunity to enter this study if no other clinical parameters precluded receiving the same chemotherapy doses during the next course of treatment. Patients entering the trial received the same chemotherapy regimen as during the previous treatment but in addition received a suspension of L-glutamine (FDA IND# 36,978), 4 gm swish and swallow orally twice a day, from day 1 of chemotherapy for 28 days or for 4 days past the resolution of any post-chemotherapy stomatitis. The suspension of glutamine was prepared by mixing 50 gm L-glutamine (supplied as a crystalline powder by Ajinomoto USA Inc., Tea Neck, N.J.), with 2 parts of ORA-Sweet (Paddock Laboratories, Minneapolis, Minn.), 1 part ORA-Plus (Paddock), and 1 part water to yield a suspension of 500 mg/ml L-glutamine. Thus the final suspension contained 500 mg/ml glutamine, 30% sucrose, 2.5% glycerin, 2.8% sorbitol, 0.04% citric acid, 0.36% $NaPO_4$, 0.16% cellulose and carboxymethylcellulose, 0.04% carrageenan, and 0.04% xanthum gum. The suspension was stored in a refrigerator until use for less than 4 weeks.

Four parameters were documented at the end of each course of chemotherapy: (1) total number of days of mucositis; (2) severity of mucositis (numbers of days at each grade); (3) the patient's subjective impression as to whether the mucositis was more severe, the same, or less severe with glutamine supplementation; and (4) the nadir neutrophil count. The severity of mucositis was graded as follows: 0, no mucositis; 1, painful mucositis not necessitating a change in oral intake; 2A, painful mucositis restricting intake to soft foods; 2B, painful mucositis restricting oral intake to liquids; and 3, mucositis preventing oral intake (modified CALGB criteria). Dietary selections (and therefore mucositis grade) were determined by the patient according to tolerance and were reported to the investigators at the end of each treatment cycle. The investigators had no input into diet decisions. Maximum grade of mucositis and total number of days of mucositis were compared by the Mann-Whitney (mucositis grade) or paired t test (days of mucositis) (InStat software; Graphpad Inc., San Diego, Calif.). All patients gave written informed consent, and the trial was approved by the Institutional Review Board of the University of Minnesota.

RESULTS

This study was initially designed as a double-blind, placebo- controlled trial in which L-aspartate, adjusted to the same pH, mixed in the same vehicle as the glutamine, was used as the placebo. However, each of the first 3 patients randomized to the placebo refused to continue the study because the placebo did not ameliorate their mucositis and they were unwilling to risk that the subsequent course of chemotherapy might be associated with mucositis with discomfort similar to that they had experienced during the previous two treatments. These 3 patients are not further described in this report. Thus the

study design was changed to that described above in Methods.

Fourteen patients, 8 male and 6 female, were entered in the study. All patients received the same chemotherapy regimen in the same doses with and without glutamine supplementation. Eight patients (patients 1 through 8) were given a 9-day continuous infusion of doxorubicin, dacarbazine, and cyclophosphamide (starting doses: 60 mg/m², 1000 mg/m², and 250 mg/m², respectively) with a wearable pump in the ambulatory setting as previously described[13]; 7 of these had a soft tissue sarcoma and 1 a mesothelioma. One patient (patient 9) with AIDS and Kaposi's sarcoma was given doxorubicin (45 mg/m²) by constant intravenous infusion over 5 days; dacarbazine (400 mg/m²), intravenous bolus; and vincristine (2 mg) intravenous push. One patient with breast cancer (patient 10) was given doxorubicin (60 mg/m²), cyclophosphamide (600 mg/m²), and 5-fluorouracil (600 mg/m²), intravenous bolus. Patient 11 received 75 mg/m² of doxorubicin over 3 days by continuous infusion and 1.5 gm/m²/day of ifosfamide by bolus administration on days 1, 2, 3, 4, and 5 for osteosarcoma. Patient 12, with a soft tissue sarcoma, received 50 mg/m²/day of etoposide orally for 21 days. Patient 13 received 7.5 gm/m² of ifosfamide and 500 mg/m² of VP-16 over 5 days and 900 mg/m² of carboplatin over 2 days by bolus infusion for an osteosarcoma. Patient 14, with squamous cell carcinoma of the head and neck, received 55 mg/m² of doxorubicin and 25 U of bleomycin by continuous infusion over 5 days.

The results of oral glutamine supplementation by grade and severity of stomatitis are summarized in Table I. The maximum grade of mucositis decreased in 12 of 14 patients with glutamine supplementation (median score 2A vs 0.5, $p < 0.001$) and remained the same (grade 1) in 2 of 14 patients (the median score of 0.5 is arbitrary, because half of the patients had a score of 1 and half a score of 0). Similarly, the total number of days of stomatitis decreased in 13 of 14 patients with glutamine supplementation (2.7 ± 0.8 (mean ± SEM) vs 9.9 ± 1.1, $p < 0.001$). Thirteen of the 14 patients felt that the mucositis was less severe with the addition of glutamine. Although this was a subjective measurement, the addition of glutamine allowed the use of chemotherapy doses that otherwise would have to have been reduced because of mucositis in 8 patients. One patient did not want another course of glutamine supplementation because she had insulin-dependent diabetes and she felt that the supplementation with the sucrose-containing suspension caused a small rise

Table I. Effect of glutamine on severity and duration of chemotherapy-associated stomatitis

Glutamine	Maximum grade*		Total number of days > grade 0†		Subjective improvement‡
	−	+	−	+	
Patient number					
1	2B	0	7	0	Yes
2	1	0	12	0	Yes
3	1	1	8	7	No
4	2B	0	7	0	Yes
5	2B	1	10	8	Yes
6	2B	1	9	4	Yes
7	1	1	14	6	Yes
8	2A	1	18	6	Yes
9	2A	1	6	3	Yes
10	2A	1	10	4	Yes
11	2A	0	7	0	Yes
12	2A	0	16	0	Yes
13	1	0	3	0	Yes
14	3	0	11	0	Yes

*Maximum grade of mucositis (modified CALGB scale) during the month following chemotherapy. The severity of mucositis was graded as: 0, no mucositis; 1, painful mucositis not necessitating a change in oral intake; 2A, painful mucositis restricting intake to soft foods; 2B, painful mucositis restricting oral intake to liquids; and 3, mucositis preventing oral intake.
†Total number of days of mucositis of all grades >0 during the month following chemotherapy.
‡Patient's subjective interpretation of whether amino acid supplementation decreased the severity of mucositis.

in her urine glucose level. No change in the nadir neutrophil count was noted with the addition of glutamine.

Four patients received more than 1 course of chemotherapy with glutamine supplementation. A beneficial effect of glutamine appeared to persist in subsequent treatments in all 4 patients in that no dose attenuations were implemented in response to mucositis. In two patients (patients 1 and 8) receiving glutamine, a subsequent increase in chemotherapy dose was administered with less mucositis than at the lower pre-glutamine chemotherapy dose.

DISCUSSION

This report demonstrates that simple oral supplementation with glutamine can significantly decrease the severity of chemotherapy-induced stomatitis in ambulatory patients. This conclusion, however, must be qualified with the realization that some placebo effect may be present, because this was not a randomized double-blind study. We would expect that the potential placebo effect would not downgrade the mucositis score from a non-zero score to zero (no mucositis) or from a 2B to a 1, but because of the subjective nature of the scale, a change from 2B to 2A or 2A to 1 could represent a placebo effect.

When the beneficial effect of glutamine is considered, it should be noted that the severity of mucositis typically increases with subsequent courses of infusional doxorubicin-based chemotherapy.[13] Thus, although the severity of stomatitis was less with the addition of glutamine in the patients described in this report, in the absence of glutamine it would have been expected to be somewhat worse. In two of four patients that received more than one course of glutamine, the chemotherapy dose was increased without the development of more severe mucositis than occurred with the lower chemotherapy dose without glutamine, a result that would be unexpected in the absence of glutamine. This beneficial effect was seen in the absence of any detectable toxicity of the glutamine. One patient with insulin-dependent diabetes noted an increase in urinary glucose level. The glutamine suspension used in this study contained 30 gm sucrose and 2.5 gm glycerin per 100 ml in addition to a small amount of fiber. We have prepared equally palatable suspensions with aspartame, and this preparation might be most appropriate when limitation of the oral intake of sugars is desired. Because the placebo control group was dropped, we cannot exclude the possibility that the vehicle itself is responsible for the observed benefits. We believe that this is unlikely, because a variety of soothing oral preparations—some of which contain sucrose and/or glycerin—have not seemed to ameliorate mucositis in our clinical experience. It is interesting to note that the one patient who experienced no benefit from the addition of glutamine had mild (grade 1) mucositis and forced herself to eat normally; perhaps the glutamine supplementation was only a small change in the oral intake of glutamine and other nutrients from her diet. In this regard, animal studies have suggested that a normal diet is more protective of the gut than glutamine alone.[2] In the final analysis, it may be that enteral supplements as close to a normal diet as possible may provide the optimal gut protection. If this is true, it may be that supplemental glutamine would be of little value in patients with normal oral dietary intake.

There has been no effective treatment for the prevention of chemotherapy-induced or radiation therapy–induced stomatitis or esophagitis, although it has been suggested that the simple maneuver of cooling the mouth by sucking ice chips may decrease the oral mucositis associated with a regimen containing 5-fluorouracil and leucovorin.[14] Although this maneuver clearly warrants further evaluation in the setting of bolus chemotherapy, it is not likely to be a viable option for continuous infusion therapy,

an increasingly popular form of therapy. A recent study demonstrated that the topical application of transforming growth factor β3 to the hamster cheek pouch at the time of chemotherapy administration decreased the severity of stomatitis; however, additional administration of transforming growth factor β3 on day 5 and day 7 eliminated this beneficial effect, presumably by inhibiting the healing process.[15] Although hypothesized to be beneficial, the use of chlorhexidine mouthwashes in patients undergoing radiation therapy to the oral mucosa was recently found to be detrimental.[16]

Several other studies have recently examined the effect of glutamine on stomatitis. In one recent study, 45 adults undergoing allogeneic bone marrow transplantation for hematologic malignancies were randomized to parenteral nutrition with or without parenteral glutamine supplementation. Significant benefits were seen in the glutamine treatment group, including a better nitrogen balance, fewer episodes of clinical infections, and a shorter hospitalization time.[4] However, no difference in the severity of oral mucositis (stomatitis) was seen. Two other recent trials of total parenteral nutrition with or without glutamine in the setting of bone marrow transplantation or high-dose chemotherapy have also not found an effect of added parenteral glutamine on oral stomatitis or esophagitis.[17,18] These studies suggest that simply providing glutamine systemically does not have a dramatic beneficial effect on oral and esophageal stomatitis. One recent study with oral glutamine also did not find a beneficial effect of stomatitis[19]; possible reasons included the use of a lower glutamine concentration, resulting in less optimal kinetics of local absorption; a shorter course of glutamine; and a small sample size.

As with all treatments designed to decrease the toxicity of cancer chemotherapy to the host, the possibility that such treatments might also protect the tumor or even enhance tumor growth must be considered (reviewed in references 1, 7, and 20). Because glutamine is a component of the normal diet, this should be of less concern than with synthetic chemoprotective agents or growth factors. In addition, the oral administration of glutamine takes advantage of the natural ability of the gut mucosa to extract a large portion of the glutamine at its desired site of action before it enters the portal circulation. Tumor cells, like normal cells, need a source of energy. The phenomenon of cancer cachexia, a common manifestation of malignancy, may be a protective response of the host to limit the potential sources of energy to the tumor. Tumor growth associated with parenteral nutrition in animals has

been demonstrated.[20-23] Some human trials have also demonstrated a shortened survival and lower response rate to chemotherapy in patients with cancer who were receiving parenteral nutrition.[20] Although glutamine utilization by tumor cells as an energy source has been documented (reviewed in references 1 and 7), two points suggest that a beneficial therapeutic ratio of glutamine might be attainable. First, the beneficial effects of glutamine were seen in this study at doses that make small contributions to the total daily caloric intake. Second, and perhaps more important, the administration of glutamine orally allows the delivery of the glutamine locally to the desired tissue where it may be utilized immediately without entering the blood and thus may not be available to the tumor. It is of interest to note that no amelioration of myelosuppression by glutamine was detected as determined by examination of the nadir neutrophil counts.

Although chemotherapy can induce mucositis throughout the gut, the present study examined only oropharyngeal mucositis. Because most glutamine presented to the epithelium of the small intestine is absorbed and metabolized by the gut directly, the protection of the oropharyngeal mucosa raises the possibility that the oropharyngeal mucosa may also be able to absorb glutamine directly. Both animal and human studies suggest that enteral nutrition results in more normal gut function than parenteral nutrition,[24] and in the setting of major abdominal trauma, enteral nutrition appears to reduce the incidence of septic complications as compared with parenteral nutrition.[25] Animal studies suggest that enteral glutamine supplementation yields a better survival rate than parenteral supplementation when it is administered after methotrexate.[5] Although glutamine supplementation of enteral diets in animals had the desired effect in several studies,[2,3,12] one study in rats suggests that the dose of glutamine is very important to obtain this effect. The addition of 5% glutamine to rats on normal chow appeared to have negative effects on intestinal adaptation after massive small bowel resection, suggesting that high concentrations of glutamine in the diet could be detrimental.[26] Thus the dose of glutamine may be critical. Although this study utilized 4 gm twice daily, the optimal dose of glutamine to achieve the desired result is unclear.

We conclude that oral glutamine supplementation is a simple, safe, and effective way to decrease the severity of stomatitis and esophagitis induced by chemotherapy, an important cause of morbidity in the treatment of patients with cancer. The role of

glutamine in the treatment of patients receiving therapy for cancer, including the effect of dose and schedule of this nutrient in the context of other dietary intake, warrants further study.

Conflict-of-interest note: The authors have a financial interest in a company that is developing oral glutamine as a treatment for mucositis.

REFERENCES

1. Skubitz KM. Glutamine as a potential treatment for the prevention of chemotherapy induced mucositis. J Infusional Chemotherapy 1994;4:64-7.
2. Shou J, Lieberman MD, Hofmann K, et al. Dietary manipulation of methotrexate-induced enterocolitis. J Parenter Enteral Nutr 1991;15:307-12.
3. Fox AD, Kripke SA, DePaula J, Berman JM, Settle RG, Rombeau JL. Effect of a glutamine-supplemented enteral diet on methotrexate-induced enterocolitis. J Parenter Enteral Nutr 1988;12:325-31.
4. Ziegler TR, Young LS, Benfell K, et al. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. Ann Intern Med 1992; 116:821-8.
5. Alverdy JC. Effects of glutamine-supplemented diets on immunology of the gut. J Parenter Enteral Nutr 1990;14:109S-13S.
6. Souba WW, Klimberg VS, Hautamaki RD, et al. Oral glutamine reduces bacterial translocation following abdominal radiation. J Surg Res 1990;48:1-5.
7. Windmueller HG. Glutamine utilization by the small intestine. Adv Enzymol 1982;53:201-37.
8. O'Dwyer ST, Smith RJ, Kripke SA, Settle RG, Rombeau JL. New fuels for the gut. In: Romineau JL, Caldwell MD, eds. Enteral and tube feeding. Philadelphia: WB Saunders, 1990: 540-55.
9. Rombeau JL. A review of the effects of glutamine-enriched diets on experimentally induced enterocolitis. J Parenter Enteral Nutr 1990;14:100S-5S.
10. Lacey JM, Wilmore DW. Is glutamine a conditionally essential amino acid? Nutr Rev 1990;48:297-309.
11. Hwang TL, O'Dwyer ST, Smith RJ. Preservation of small bowel mucosa using glutamine enriched parenteral nutrition. Surg Forum 1987;38:56-8.
12. Klimberg VS, Souba WW, Dolson DJ, et al. Prophylactic glutamine protects the intestinal mucosa from radiation injury. Cancer 1990;66:62-8.
13. Hamdan H, Savage PD, Thompson RC Jr, Skubitz KM. A phase I study of ambulatory continuous infusion chemotherapy with cyclophosphamide, doxorubicin, and dacarbazine for soft tissue sarcomas. Journal of Infusional Chemotherapy 1992;2:97-102.
14. Mahood DJ, Dose AM, Loprinzi CL, et al. Inhibition of fluorouracil-induced stomatitis by oral cryotherapy. J Clin Oncol 1991;9:449-52.
15. Sonis ST, Lindquist L, Van Vugt A, et al. Prevention of

chemotherapy-induced ulcerative mucositis by transforming growth factor $\beta 3$[1]. Cancer Res 1994;54:1135-8.

16. Foote RL, Loprinzi CL, Frank AR, et al. Randomized trial of a chlorhexidine mouthwash for alleviation of radiation-induced mucositis. J Clin Oncol 1994;12:2630-3.

17. van Zaanen HCT, van der Lelie H, Timmer JG, Furst P, Sauerwein HP. Parenteral glutamine dipeptide supplementation does not ameliorate chemotherapy-induced toxicity. Cancer 1994;74:2879-84.

18. Schloerb P, Amare M. Total parenteral nutrition with glutamine in bone marrow transplantation and other clinical applications. J Parenter Enteral Nutr 1993;17:407-13.

19. Jebb SA, Osborne RJ, Maughan TS, et al. 5-Fluorouracil and folinic acid–induced mucositis: no effect of oral glutamine supplementation. Br J Cancer 1994;70:732-5.

20. McGeer AJ, Detsky AS, O'Rourke K. Parenteral nutrition in patients receiving cancer chemotherapy. Ann Intern Med 1989;110:734-6.

21. Fischer JE. Adjuvant parenteral nutrition in the patient with cancer. Surgery 1984;96:578-80.

22. Fischer JE, Chance WT. Total parenteral nutrition, glutamine, and tumor growth. J Parenter Enteral Nutr 1990;14:86S-9S.

23. Steiger E, Oram-Smith J, Miller E, Kuo L, Vars HM. Effects of nutrition on tumor growth and tolerance to chemotherapy. J Surg Res 1975;18:455-61.

24. Fong Y, Marano MA, Barber A, et al. Total parenteral nutrition and bowel rest modify the metabolic response to endotoxin in humans. Ann Surg 1989;210:449-57.

25. Moore FA, Moore EE, Jones TN, McCroskey BL, Peterson VM. TEN versus TPN following major abdominal trauma-reduced septic morbidity. J Trauma 1989;29:916-23.

26. Vanderhoof JA, Blackwood DJ, Mohammadpour BS, Park JHY. Effects of oral supplementation of glutamine on small intestinal mucosal mass following resection. J Am Coll Nutr 1992;11:223-7.