148-6071/99/2301-0007$03.00/0
JOURNAL OF PARENTERAL AND ENTERAL NUTRITION
Vol. 23, No. 1
Printed in U.S.A.

Original Communications

Effect of Long-Term Oral Glutamine Supplements on Small Intestinal Permeability in Patients With Crohn's Disease

Elly Den Hond, PhD; Martin Hiele, MD, PhD; Marc Peeters, MD, PhD; Yvo Ghoos, PhD; and Paul Rutgeerts, MD, PhD

From the Department of Gastroenterology, University Hospital Leuven, Belgium

ABSTRACT. *Background:* Glutamine is a major fuel and an important nitrogen source for the small intestinal cell. It plays a key role in maintaining mucosal cell integrity and gut barrier function. Increased permeability may be a factor in the pathogenesis of Crohn's disease and may be an interesting parameter in the follow-up of the disease. Therefore, the aim of this study was to examine whether oral glutamine supplements are able to restore an increased intestinal permeability in patients with Crohn's disease. *Methods:* The inclusion criteria for the study were Crohn's disease and a disturbed small intestinal permeability for ^{51}Cr-EDTA. Of 38 patients screened, 18 had an increased permeability (6 hours urinary excretion >1.1% of label recovered in urine). Fourteen patients were included in the study and were randomized to receive either oral glutamine (7 g three times per day; $n = 7$) or placebo (7 g glycine three times per day; $n = 7$) in addition to their normal treatment during a 4-week period. The study was performed in a double-blind manner. *Results:* Baseline permeability (mean ± SD) was 2.32% ± 0.77% dose in the glutamine group and 2.29% ± 0.67% dose in the placebo group. Permeability did not change significantly after glutamine (3.26% ± 2.15% dose) or after placebo (2.27% ± 1.32% dose). There was no significant effect on plasma glutamine, plasma glutamate, plasma ammonium, Crohn's disease activity index, C-reactive protein, or nutritional status. *Conclusions:* Oral glutamine supplements, in the dose administered, do not seem to restore impaired permeability in patients with Crohn's disease. (*Journal of Parenteral and Enteral Nutrition* **23**:7–11, 1999)

Glutamine has an important function in supporting the healthy gut,[1] but its role becomes still more pronounced if the intestinal cell is stressed (eg, in the postoperative period, during starvation, in advanced malignant disease, in sepsis, or during inflammation).[2] In a clinical trial, van der Hulst et al[3] showed that addition of glutamine to total parenteral nutrition (TPN) can prevent an increase of small intestinal permeability. In a study with healthy volunteers, Buchman et al[4] demonstrated that after a 14-day period of TPN, refeeding with glutamine- and arginine-supplemented enteral nutrition was superior to standard enteral nutrition to restore permeability.

Approximately 50% of patients with Crohn's disease have an increased small intestinal permeability. The fact that permeability also is increased in healthy first-degree relatives[5,6] suggests that permeability is not just a result of inflammation but that it may precede the disease and be involved in the pathogenesis. Furthermore, in patients with quiescent disease, increased permeability seems a good predictor of relapse in the following year.[7]

Received for publication, December 31, 1997.
Accepted for publication, August 11, 1998.
Correspondence and reprint requests: Paul Rutgeerts, MD, PhD, Department of Gastroenterology, University Hospital Leuven, Herestraat 49, B-3000 Leuven, Belgium.

The aim was to study whether glutamine supplements can lower increased permeability in patients with Crohn's disease in a double-blinded, randomized, placebo-controlled study.

METHODS

Inclusion of Patients

Thirty-eight patients with Crohn's disease were selected to perform a permeability test. Patients were recruited from both the gastroenterology ward and the outpatient clinic. No selection was made for degree or localization of the disease. Small intestinal permeability was measured using ^{51}Cr-EDTA (Amersham International, Amersham, United Kingdom) as a permeability probe. After an overnight fast, the patient drank 160 mL Nutridrink (Nutricia, Bornem, Belgium) to which 50 μCi ^{51}Cr-EDTA was added; the glass was rinsed with 90 mL water.[8] Eating or drinking was not allowed for the next 2 hours, and urine was collected for the next 6 hours. Volumes of urine were recorded, and 1-mL aliquots were counted for radioactivity by a β liquid scintillation counter (model 4430; Packard, Downers Grove, IL) within 48 hours after sampling. Results were expressed as the percentage of the orally administered dose of ^{51}Cr-EDTA. Increased permeability was defined as a 6-hour cumulative excretion totaling >1.1% of the dose. Of the 38 patients who underwent a permeability test, 18 patients had increased permeability. Of these 18 patients, 4 were not included

in the study: 2 patients had surgery, 1 patient had renal problems that were considered a possible contraindication to receiving large doses of amino acids, and 1 refused further cooperation. The remaining 14 patients were included and completed the study.

Clinical Trial

Fourteen patients with increased permeability were randomly assigned to receive glutamine (ICN Biomedicals Inc, Cleveland, OH) or placebo, which in this case was glycine (ICN Biomedicals Inc, Aurora, OH). Both amino acids have a neutral taste and are powders that can easily be dissolved in water. Both the patients and the investigators were blinded to the code of the study. Patients were instructed to ingest 7 g glutamine (or placebo) in water three times daily during a 4-week period. The study was conducted at home, and the patients were asked to follow their normal diet and to keep all medication at a constant dose. Each patient kept a daily diary for glutamine intake, clinical activity of the disease, and medication. Patients were contacted once by telephone to check for motivation and to answer possible questions.

The choice of dose (21 g glutamine/d) was based on clinical trials with TPN,[9–12] in which between 0.2 and 0.6 g glutamine/kg body wt was administered. For a mean body weight of 60 kg, this would mean a dose between 12 and 36 g glutamine. Rather than constructing a dose–response curve, we decided to use one relatively high dose of glutamine (21 g is approximately one third of protein needs). It is unlikely that lower doses of glutamine show a better effect, but higher doses would be difficult to integrate into the patient's daily life.

In spite of the fact that a normal diet contains glutamine, food intake of the patients was not standardized, but the patients were instructed to follow their normal diet because we wanted to study the effects of large supplements of glutamine in comparison with placebo, independent of the normal protein intake.

Evaluation of Effect

Before and immediately after the study period, the following parameters were measured.

Small intestinal permeability was measured as described previously.

The Crohn's disease activity index (CDAI), a measure for the clinical condition of the patient, was calculated.[13]

Glutamine, glutamate, and ammonium in plasma were measured enzymatically. The principle of the three measurements is the conversion of the reduced form of nicotinamide-adenine dinucleotide (NADH) from and to its oxidized form (NAD^+). The ratio of NADH to NAD^+ was followed by a spectrophotometer. Blood samples were centrifuged during 15 minutes at 3000 rpm. Ammonium was measured within 1 hour with a test kit (Boehringer Mannheim GmbH, Mannheim, Germany). Plasma samples for glutamine and glutamate were prepared as follows. The protein in 2 mL plasma was precipitated with 2 mL perchloric acid 30% and centrifuged for 10 minutes at 3000 rpm. The supernatant was decanted and collected. The precipitate was washed with .5 mL perchloric acid 30% and again centrifuged for 10 minutes at 3000 rpm. The supernatants were pooled, and 2 mL buffer (KOH-KCl-hydroxine) was added. The pH was adjusted with KOH 30% to a value between 6 and 7. The samples were stored on ice for 10 minutes and then were centrifuged for 10 minutes at 3000 rpm. Glutamate[14] and glutamine[15] in the supernatant were measured enzymatically according to the method described by Bergmeyer.

The nutritional status of the patients was evaluated by height, weight, percentage of ideal body weight (reference weight was taken from the 1983 Metropolitan Height-Weight Tables), body mass index ([BMI] weight [kg]/height^2 [m]), triceps skinfold (TSF), midarm muscle arm circumference ([MMAC] 3.14 $\times$ TSF), serum albumin, and serum transferrin.

Liver function tests were performed as a check-up because the patients received considerable supplements of amino acid (21 g/d).

Statistical Analysis

The disease characteristics and the clinical parameters at baseline were compared between the glutamine group and the placebo group. Means ($\pm$ SD) were compared by Student's t test (PROC TTEST; SAS Institute, Cary, NC); proportions were compared by a χ^2 test (PROC FREQ; SAS Institute). To compare the effect of glutamine and placebo on clinical outcome, the difference before and after treatment in both groups was compared by means of Student's t test (PROC TTEST; SAS Institute).

Ethics

Informed consent was obtained from all patients, and the study was approved by the Ethical Committee of the University Hospital.

RESULTS

Table I shows the anthropometric data in the glutamine group and the placebo group. These data are purely descriptive because the groups were too small for statistical comparison, especially because anthropometric data of men and women should be considered separately. The disease characteristics are also shown in Table I. Patients were well matched for duration of disease (t test: $p = .14$), type of disease (χ^2: $p = .17$), medication (χ^2: $p = .40$), and number of operations (χ^2: $p = .57$).

According to the patients' diaries, patient compliance was good. Although there was no objective way to control for glutamine intake, all patients were strongly motivated to cooperate. They reported a maximum of 9 missed doses of 84 total doses; these doses were missed for practical reasons or because of forgetfulness.

Table II shows parameters studied before and after treatment with either glutamine or placebo.

The baseline permeability did not differ significantly between the two treatment groups, and there was no effect from either glutamine treatment or placebo

TABLE I
Anthropometric data and disease characteristics of patients in the group receiving glutamine and in the group receiving placebo

	Glutamine (n = 7)	Placebo (n = 7)
Age (y)*	25.0 ± 7.9	38.2 ± 13.4
Sex (M/F)	1/6	3/4
Weight (kg)		
Men*	89.0	69.8 ± 11.3
Women*	46.2 ± 3.9	62.5 ± 10.9
% Ideal body weight		
Men*	130	97 ± 4
Women*	86 ± 11	105 ± 12
Height (m)		
Men*	1.72	1.76 ± 0.09
Women*	1.62 ± 0.07	1.62 ± 0.02
Body mass index (kg/m^2)		
Men*	30.1	22.3 ± 1.4
Women*	17.8 ± 2.5	23.8 ± 3.6
Skinfold thickness (mm)		
Men*	19.2	11.0 ± 1.7
Women*	10.8 ± 2.8	23.5 ± 4.0
MAMC (cm)		
Men*	27.9	24.9 ± 1.7
Women*	18.8 ± 1.9	21.9 ± 2.9
Duration of disease (y)*	5.4 ± 6.0	8.9 ± 6.8
Type†		
Terminal ileum	6	2
Ileocolitis	1	2
Pancolitis	0	3
Medications†		
Salicylates	4	5
Corticosteroids	5	3
Immunosuppresiva	2	1
Antibiotics	0	2
Number of operations†		
0	4	3
1	3	3
2	0	1

M, male; F, female; MAMC, midarm muscle circumference.
*Values are means ± SD.
†Values are number of patients.

treatment on permeability. Individual changes in permeability are shown in Figure 1.

The mean CDAI in the two treatment groups did not differ at baseline and was not affected by either glutamine or placebo. The mean percent change after treatment was +8% of the baseline value in the group receiving glutamine (individual values ranging from −59% to +67%) and 0% in the group receiving glycine (range, −45% to +63%). Figure 2 shows the individual values for CDAI.

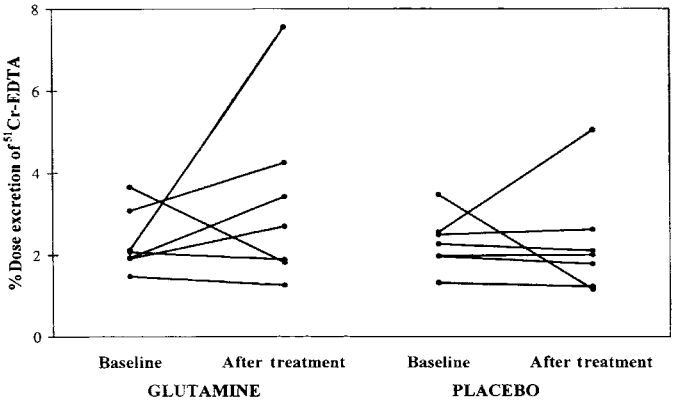

FIG. 1. Permeability (urinary % dose excretion of ^{51}Cr-EDTA) before and after treatment in patients receiving glutamine and in patients receiving placebo.

No significant differences in baseline C-reactive protein (CRP) or changes in CRP were found. If 0.80 mg/dL is set as normal limit, four of seven patients in the group receiving glutamine had normal values before the study, compared with one patient after the study. In the group receiving glycine, four of seven patients had normal values at baseline, and two patients retained a normal CRP after the treatment.

Although there was a slight increase in plasma glutamine after treatment, this difference was not significant. Both plasma glutamate and ammonium remained stable.

Serum albumin and transferrin, parameters that are often used as indicators for nutritional status, did not change significantly during the study. Other nutritional parameters such as weight, height, BMI, TSF, and MMAC did not change significantly, neither in the glutamine group nor in the placebo group (data not shown).

Liver function tests were not significantly affected, implying that the dose of amino acid used in this study was not hepatotoxic.

DISCUSSION

Glutamine is a major fuel and an important nitrogen source for the small intestinal cell. Approximately 50%

TABLE II
Clinical parameters before and after study in the group receiving glutamine and in the group receiving placebo

	Glutamine		Placebo		*p* values	
	Baseline	After treatment	Baseline	After treatment	Baseline glutamine *vs* baseline placebo*	Difference glutamine *vs* difference placebo‡
Permeability (% dose)	2.32 ± 0.77	3.26 ± 2.15	2.29 ± 0.67	2.27 ± 1.32	.93	.36
Crohn's disease activity index	170 ± 99	163 ± 103	115 ± 69	106 ± 74	.25	.93
C-reactive protein (mg/dL)	1.5 ± 1.0	1.4 ± 1.2	0.5 ± 0.5	1.4 ± 1.6	.09	.18
Plasma glutamine (mmol/L)	0.485 ± 0.079	0.608 ± 0.163	0.524 ± 0.061	0.494 ± 0.028	.49	.13
Plasma glutamate (mmol/L)	0.058 ± 0.030	0.056 ± 0.020	0.052 ± 0.024	0.054 ± 0.025	.80	.88
Plasma ammonia (mmol/L)	0.040 ± 0.014	0.038 ± 0.020	0.049 ± 0.031	0.043 ± 0.020	.49	.80
Serum albumin (g/dL)	3.86 ± 0.54	4.23 ± 0.45	4.10 ± 0.47	4.03 ± 0.17	.49	.49
Serum transferrin (mg/dL)	284 ± 36	321 ± 27	277 ± 78	357 ± 94	.89	.51

Values are means ± SD.
*Baseline values between both groups are compared to check if patients are well matched.
‡Differences before and after treatment in both groups are compared to know the effect of glutamine in comparison to placebo.

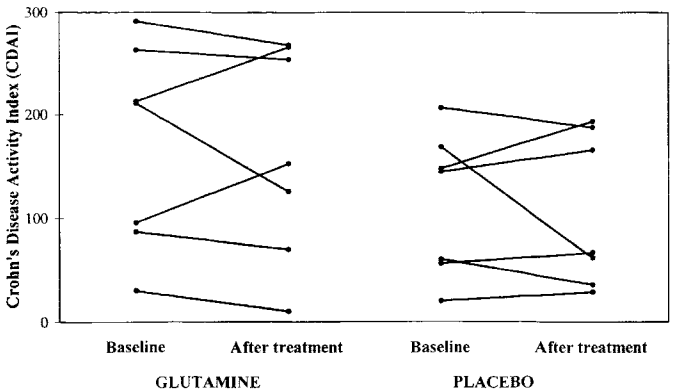

FIG. 2. Crohn's disease activity index before and after treatment in patients receiving glutamine and in patients receiving placebo

of the carbon of glutamine that is administered through the lumen is oxidized to carbon dioxide by the intestinal cell, and 38% of the nitrogen is released as ammonia.[1] The splanchnic glutamine metabolism plays a major role in several pathophysiological conditions, such as operative stress, starvation, advanced malignant disease, sepsis, endotoxemia, and inflammation.[2]

Numerous clinical studies have shown a beneficial effect of glutamine added to TPN. The studies concerned patients after bone marrow transplantation[9–11] or after abdominal surgery[12,16] and showed general effects, such as a shorter hospital stay,[10,11] fewer infections,[9,10] a faster normalization of the nitrogen balance,[10,12,16] and a dramatic decrease of intracellular glutamine concentration after glutamine-supplemented TPN.[12,16] Studies in animals showed a positive effect of glutamine added to TPN on gastrointestinal integrity (eg, on villus height, crypt cell production rate, and mucosal protein concentration).[17–19] Several studies in rats (all TPN)[20–22] and two studies in humans (one with TPN in patients[3] and one with enteral nutrition in healthy volunteers[4]) specifically investigated the relationship between glutamine and small intestinal permeability. All studies showed that supplements of glutamine can prevent an increase of permeability compared with standard nutrition. In an uncontrolled study, Zoli et al[23]described that oral glutamine supplements were able to decrease permeability in Crohn's disease.

Intestinal permeability is assessed noninvasively *in vivo* by measuring urinary excretion of orally administered test substances. Most commonly used test probes are polymers of polyethylene glycol, ^{51}Cr-EDTA, and the lactulose-manntiol ratio. In our study, ^{51}Cr-EDTA was used because sample preparation is easy and samples could be measured overnight. Obtaining a quick result was necessary because increased permeability was an inclusion criteria for the study. The use of ^{51}Cr-EDTA as a permeability probe is similar to using oligosaccharides such as lactulose.[24]

Small intestinal permeability is increased in Crohn's disease,[25] but it remains unclear whether this is a secondary result of inflammation or whether it is due to a primary genetic abnormality. Increased permeability in healthy relatives of patients with Crohn's disease[5,6] suggests that it may be involved in disease pathogenesis. In patients with quiescent disease, the finding that increased permeability was a good predictor of relapse in the following year[7] also suggests that increased permeability precedes active disease. If this is true, controlling permeability would be of major importance in the treatment of Crohn's disease.

In this study, we examined the effect of oral glutamine supplements on intestinal permeability in Crohn's disease. Seven grams of glutamine, three times daily during a 4-week period, did not result in a significant decrease of permeability in patients with increased basal values nor was there a significant effect on other parameters, such as CDAI, CRP, plasma glutamine, plasma glutamate, plasma ammonium, or nutritional indices.

The reasons for the lack of effect are unclear. A major problem is the absorption of glutamine. We do not know which part of the dose is decomposed (eg, by gastric acid) or how fast glutamine is absorbed in the lumen. This problem is relevant because Crohn's disease is mainly a disease of the ileum. Yet, two clinical trials with enteral doses[4,23] showed positive results, assuming that enteral doses can be effective. Another problem is the dose and time of administration. Because we do not know what mechanism initiates the increase of permeability, it is impossible to say what the best moment is to administer glutamine. The study that mostly resembles the design of our study was published by Zoli et al[23] and describes a normalization of increased permeability in Crohn's disease by oral glutamine supplements. However, these results were obtained in an open trial and were only published in abstract form; therefore, it is impossible to conduct a detailed comparison to our results.

In conclusion, a double-blinded, randomized, placebo-controlled study was performed to investigate the effect of glutamine on small intestinal permeability in Crohn's disease patients. Oral glutamine supplements were not able to normalize increased permeability.

ACKNOWLEDGMENT

The study was supported by a Clinical Research in Human Nutrition grant from the Institute Danone, Brussels, Belgium.

REFERENCES

1. Windmueller HG: Glutamine utilization by the small intestine. Adv Enzymol 53:201–237, 1982
2. Souba WW, Klimberg VS, Plumley DA, et al: The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. J Surg Res 48:383–391, 1990
3. van der Hulst R, van Kreel BK, von Meyenfeldt MF, et al.: Glutamine and the preservation of gut integrity. Lancet 341: 1363–1365, 1993
4. Buchman AL, Moukarzel AA, Bhuta S, et al.: Parenteral nutrition is associated with intestinal morphologic and functional changes in humans. JPEN 19:453–460, 1995
5. Hollander D, Vadheim CM, Brettholz E, et al.: Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med 105:883–885, 1986

6. Peeters M, Nevens H, Baert F, et al.: Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. Gastroenterology 111:597–603, 1996
7. Wyatt J, Vogelsang H, Hubl W, et al.: Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet 341: 1437–1439, 1993
8. Peeters M, Hiele M, Ghoos Y, et al.: Test conditions greatly influence permeation of water soluble molecules through the intestinal mucosa: need for standardisation. Gut 35:1404–1408, 1994
9. Scheltinga MR, Young LS, Benfell K, et al.: Glutamine-enriched intravenous feedings attenuate extracellular fluid expansion after a standard stress. Ann Surg 214:385–395, 1991
10. Ziegler TR, Young LS, Benfell K, et al.: Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. Ann Int Med 116:821–828, 1992
11. Schloerb PR, Amare M: Total parenteral nutrition with glutamine in bone marrow transplantation and other clinical applications (a randomized, double-blind study). JPEN 17:407–413, 1992
12. Hammarqvist F, Wernerman J, Ali R, et al.: Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall of muscle protein synthesis and improves nitrogen balance. Ann Surg 209:455–461, 1989
13. Best WR, Becktel JM, Singleton JW, et al.: Development of a Crohn's disease activity index. Gastroenterology 70:439–444, 1976
14. Bernt E, Bergmeyer HU: L-glutamat. UV-test mit glutamatdehydrogenase und NAD. IN Methoden der enzymatische Analyse, herausgegeben von Hans Ulrich Bergmeyer. Verlag Chemie GmbH, Weinheim, Germany, 1974, pp 1749–1753
15. Lund P: L-glutamin. Bestimmung mit glutaminase und glutamat-dehydrogenase. IN Methoden der enzymatische Analyse, herausgegeben von Hans Ulrich Bergmeyer. Verlag Chemie GmbH, Weinheim, Germany, 1974, pp 1767–1769
16. Stehle P, Zander J, Mertes N, et al.: Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. Lancet I:231–233, 1989
17. Platelli C, McCauley R, McCulloch R, et al.: The influence of parenteral glutamine and branced-chain amino acids on total parenteral nutrition-induced atrophy of the gut. JPEN 17:348–354, 1993
18. Klimberg VS, Souba WW, Sitren H, et al.: Glutamine-enriched total parenteral nutrition supports gut metabolism. Surg Forum 40:175–177, 1989
19. Chen K, Okuma T, Okamura K, et al.: Glutamine-supplemented parenteral nutrition improves gut mucosa integrity and function in endotoxemic rats. JPEN 18:167–171, 1994
20. Li J, Langkamp-Henken B, Suzuki K, et al.: Glutamine prevents parenteral nutrition-induced increases in intestinal permeability. JPEN 18:303–307, 1994
21. Dugan ME, McBurney MI: Luminal glutamine perfusion alters endotoxin-related changes in ileal permeability of the piglet. JPEN 19:83–87, 1995
22. Scheppach W, Dusel G, Kuhn T, et al.: Effect of L-glutmaine and n-butyrate on the restitution of rat colonic mucosa after acid induced injury. Gut 38:878–885, 1996
23. Zoli G, Care M, Falco F, et al.: Effect of oral glutamine on intestinal permeability and nutritional status in Crohn's disease. Gastroenterology 108:A766, 1994
24. Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. Gastroenterology 108:1566–1581, 1995
25. Bjarnason I, O'Morain C, Levi AJ, et al.: Absorption of ^{51}Chromium-labeled EDTA in inflammatory bowel disease. Gastroenterology 85:318–322, 1983