Journal of Parental & Enteral Nutrition. 2013;20(10):1-9

Efficacy of glutamine in the prevention of acute radiation enteritis: a randomized controlled trial

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Abstract

Background: Acute radiation enteritis is a common adverse effect related to radiotherapy (RT). Glutamine is an immune modulator and antioxidant amino acid that can exert a protective role in patients receiving abdominal or pelvic radiation. The aim of this study was to test if glutamine prevents radiation enteritis during RT. *Materials and Methods*: Double-blind, randomized, controlled trial including 69 patients who needed RT because of pelvic or abdominal malignancies and received glutamine (30 g/d) or placebo (casein, 30 g/d). Enteritis was evaluated according to the Radiation Therapy Oncology Group scale, intestinal inflammation using fecal calprotectin, and gut integrity with citrulline. The incidence of enteritis was analyzed by Kaplan-Meier curves, and the hazard ratio (HR) was calculated using Cox regression. *Results*: Patients were predominantly male (65.2%), with an average (SD) age of 66.6 (9.9) years, with urologic (44.9%), rectal (24.6%), or gynecological cancer (23.1%). More patients developed enteritis with glutamine than with the placebo (55.9% vs 22.0%; P = .002), with an HR of 1.59 (95% confidence interval, 0.62–4.05). There were no differences in final calprotectin levels (glutamine, 57.9 [85.8] mg/kg vs placebo, 54.0 [57.7] mg/kg; P = .182) or the number of patients with values >50 mg/kg (glutamine, 58.1% vs placebo, 54.6%; P = .777). Final citrulline levels were similar between groups (glutamine, 26.31 [10.29] mmol/L vs placebo, 27.69 [12.31] mmol/L; P = .639), without differences in the number of patients with <20 mmol/L (glutamine, 24.1% vs placebo, 25.0%; P = .938). Citrulline concentration was reduced during RT with placebo but remained unchanged with glutamine. *Conclusion*: Glutamine does not prevent the development of enteritis during RT.

Keywords

Glutamine; Acute radiation enteritis; Citrulline; Calprotectin; Nitric oxide

Clinical Relevancy Statement

Toxicity related to cancer therapy negatively influences quality of life of patients, compromises the completion of treatments, and is associated with higher expenses. Glutamine may have a protective effect in patients receiving radiation therapy, but few randomized controlled trials have been published testing this hypothesis. The findings of this study do not support the use of glutamine in the prevention of acute radiation enteritis and could even promote the development of intestinal toxicity.

Introduction

Despite the development of new therapies for the treatment of cancer, approximately half of oncology patients receive radiotherapy (RT), including those with abdominal and pelvic neoplasias. Nevertheless, the therapeutic use of ionizing radiation is limited by its adverse effects. Acute radiation-related toxicity depends on the suppression of cell replication in organs of rapid cell turnover (eg, bone marrow), typically develops during its administration, and is resolved within 1–2 months. Acute radiation enteritis (ARE) represents the toxicity that develops in the small intestine during RT. ARE involves different mechanisms, including the depletion of stem cells in the crypts of Lieberkühn, endothelial dysfunction, alterations in intestinal contractility, and changes that radiation induces on intestinal microbiota.[1] These alterations contribute to a decrease in the absorptive surface of the mucosa and to an increase in the speed of intestinal transit, resulting in diarrhea, abdominal pain, anorexia, nausea, and vomiting.

Ionizing radiation causes cellular damage by means of several mechanisms, including oxidative stress (OS), inflammation, genetic damage, apoptosis, changes in the cellular membrane, and vascular alterations. Acute exposure to radiation generates free radicals, such as the superoxide radical. Human cells possess various defenses against these reactive molecules, including enzymes (eg, glutathione peroxidase), cofactors (eg, glutathione), and nutrients (eg, glutamine). Both cells and tissues have a limited ability to enhance the antioxidant system in response to an oxidant factor such as radiation, and when it is overwhelmed by reactive species and free radicals, molecular damage is induced.[2] In addition to oxidative reactions, radiation stimulates the generation of nuclear factor κB (NF-κB), interleukins, and tumor necrosis factor–α (TNF-α). The gut, liver, and brain have a high expression of the former, which is only moderately produced in the lung, heart, spleen, kidney, and testes.[3]

Glutamine is the most abundant amino acid in the body.[4] Several functions of glutamine may play a role in the protection of the healthy gut during RT. First, glutamine contributes to intestinal trophism, as the small intestine is the principal consumer of glutamine in the body. Second, glutamine is the precursor of glutathione, a key molecule in the antioxidant chain. Third, glutamine modulates the inflammatory response in different immune cells and regulates cytokine production. And finally, glutamine protects cells from various insults by the production of heat shock proteins and also influences apoptosis.[5] Few studies have assessed if glutamine prevents ARE, and the results have been disparate.[6⇓-8] The aim of this study was to test if oral glutamine prevents the development of acute enteritis, protects the intestinal mucosa, and limits gut inflammation in patients receiving either abdominal or pelvic radiation therapy.

Patients and Methods

A randomized, controlled, double-blind study was designed to compare the effectiveness of glutamine vs placebo in the prevention of ARE. The study was evaluated by the local Research Ethics Committee, which confirmed that the study conformed to the ethical and legal standards required for biomedical research according to the Declaration of Helsinki. The study was registered in Clinical Trials with the number NCT00828399.

Patients

Patients >18 years for whom RT of the abdominal/pelvic cavity was planned because of a neoplasm in that location, regardless of other cancer treatments (surgery, chemotherapy, brachytherapy), were considered suitable for the trial. All patients were recruited by the research group in the Complejo Asistencial Universitario de León, where the trial took place. Exclusion criteria included life expectancy <1 year, intestinal failure or short bowel syndrome of any etiology, relevant intestinal diseases (inflammatory bowel disease, celiac disease, Whipple disease), moderate or severe chronic kidney disease, or inability to receive oral medication or to understand the provided information. All patients received verbal and written information about the purpose and methodology of the study and were included after signing the informed consent document. Randomization, which took place at the time of recruitment, was conducted by the investigator who recruited the patients in a 1:1 ratio to each group by generating a list of random numbers with the software Epidat 3.1 (Organización Panamericanade la Salud, Washington D.C., USA; Figure 1). Patients, the principal investigator, and co-investigators were blinded for treatment assignation and outcomes until the end of the trial, and the blind was broken after the completion of statistical analysis.

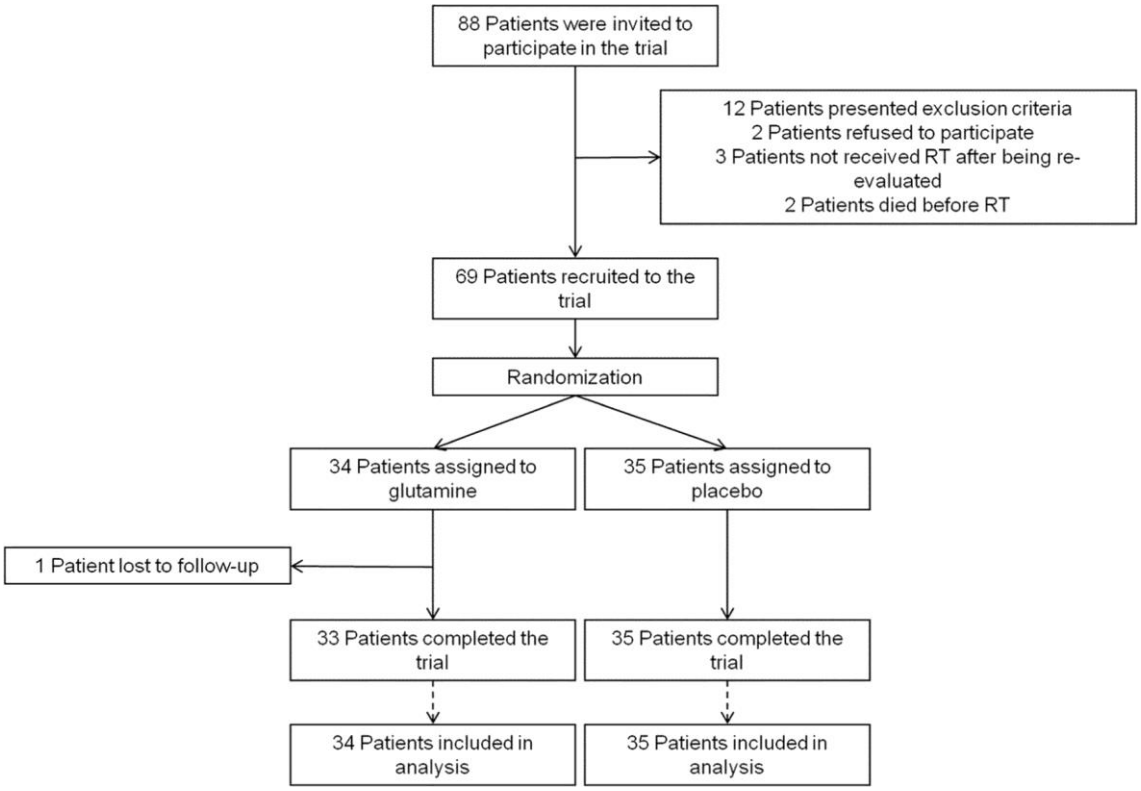

Figure 1. Flowchart of the study. The recruitment included 69 of the 88 patients initially invited to participate, and all the recruited patients were randomized and included in the intention-to-treat analysis. One patient in the glutamine group withdrew on the last visit of the study due to an underlying disease different from that indicated for radiotherapy (RT).

Intervention

The treatment group received 30 g/d of oral glutamine (Glutamina NM; Nutrición Médica, Madrid, Spain), and the placebo group received 30 g/d of pure whole casein (Proteína NM; Nutrición Médica). This dose of glutamine was selected following previous studies that administered oral glutamine for RT, chemotherapy, or bone marrow transplantation. Both substances were supplied as a powder for dissolution without flavor, contained in nonlabeled sachets of 10 g of product. Investigators recommended the consumption of 3 sachets/d, from 3 days before starting RT and to the completion of it. Each sachet was dissolved in 200 mL of water, and the solution was drunk after a meal. Both glutamine and placebo had similar color, taste, and texture before and after dissolution. Patients with mild chronic kidney disease were informed by a dietitian to adjust their dietary protein intake, taking into account the added contribution of the substance administered during the study.

Acute Radiation Enteritis Assessment

Patients were evaluated at 3 different time points during the study: before RT (recruitment), in the middle of the RT period, and after finishing RT. They were asked about the number and characteristics of stools at each visit. Intestinal toxicity was classified according to the criteria of the Radiation Therapy Oncology Group (RTOG): grade 0 (no diarrhea), grade 1 (increase of 2–3 stools/d), grade 2 (increase of 4–6 stools/d, nocturnal stools, mild cramping), grade 3 (increase of 7–9 stools/d, fecal incontinence, severe cramping), and grade 4 (increase >10 stools/d, bloody diarrhea, parenteral nutrition).

Nutrition Assessment

All patients were evaluated at each visit using the Subjective Global Assessment (SGA). Anthropometry included the measurement of height and body weight, dynamometry (Smedley's Dynamo Meter; YOII/ Tsutsumi /TTM, Tokyo, Japan), and the determination of fat-free mass (FFM) and fat mass (FM) by bioelectrical impedance (Tanita Body Composition Analyzer TBF-300; Tanita, Arlington Heights, IL). From these results, the fat-free mass index was calculated: FFMI = fat-free mass (kg)/height $(m)^2$. Dietary intake was assessed using 3-day dietary records at each visit, with the support of a dietitian, who analyzed the data using Dietsource 2.0 (Novartis Consumer Health-Cath Soft, 1997–2003; Novartis, East Hanover, NJ).

Analytical Methods

Blood samples were taken at each visit for blood cell counts, biochemistry (glucose, serum urea nitrogen, creatinine, ions, alanine aminotransferase, aspartate aminotransferase), nutrition biomarkers (serum albumin, prealbumin, retinol binding protein, and total cholesterol), and inflammatory parameters (C-reactive protein [CRP] and ferritin). Tumor markers (carcinoembryonic antigen [CEA] and prostate-specific antigen [PSA]) were measured before and after RT. Villous functional mass was assessed using plasma citrulline. For this purpose, 0.5 mL of blood was obtained before and after RT, conserved in EDTA, and analyzed by high-performance liquid chromatography. A cutoff of 20 mmol/L was established for significant intestinal mucosal damage. Inflammation of the intestinal mucosa was determined by means of fecal calprotectin, by obtaining a stool sample before and after RT, which was analyzed by enzyme-linked immunosorbent assay. Values >50 mg/g were considered to indicate gut inflammation.[9]

Statistical Analysis

A total sample size of 70 patients (35 per group) was calculated to detect a difference of 30% in the incidence of ARE among groups with an accuracy of 5%, according to the results reported by Ramírez Vargas[10] at the Congress of the European Society for Radiotherapy and Oncology in 2009. An intention-to-treat analysis was performed. The normal distribution of quantitative variables was examined by the Kolmogorov-Smirnov test. Those with a normal distribution were summarized as the mean and standard deviation (SD) and compared with an unpaired Student *t* test. When more than 2 groups were compared, the analysis of variance test was used. Quantitative variables without a normal distribution were summarized by the median (Md) and interquartile range (IQR) and compared using the Mann-Whitney *U* test or the Friedman test when comparing more than 2 groups. Categorical variables were summarized as percentages and compared with the χ^2 test. The comparison of the incidence of enteritis between treatment and placebo was performed using the Kaplan-Meier method, and survival curves were compared using the Mantel-Haenszel test. The hazard ratio (HR) was calculated using Cox regression. Multivariate analyses were performed using binary logistic and linear regression.

Results

Sixty-nine patients were recruited, of whom 34 were assigned to glutamine (GLN) and 35 to placebo (PLA). During follow-up, 1 patient in the former group dropped out the trial due to complications from an underlying disease. The characteristics of both groups are summarized in Table 1. Both treatment groups received the same dose of RT (GLN Md [IQR] = 51.0 [28.0] Gy; PLA Md [IQR] = 50.4 [29.0] Gy; P = .636), with a fractionation dose of 1.8 (IQR = 0.1) Gy/session for patients receiving GLN and 1.9 (IQR = 0.1) Gy/session for those who received PLA (P = .745). The received dose of GLN was 0.4 (0.1) g/kg/d, with a minimum dose of 0.3 g/kg/d and a maximum of 0.6 g/kg/d.

Table 1. Patient Characteristics.

	Glutamine (n = 34)	Placebo (n = 35)	*P* Value
Age, y, mean (SD)	64.9 (9.7)	68.1 (10.0)	.189
Male sex, %	64.7	65.7	.930
Weight, kg, mean (SD)	74.8 (13.7)	68.9 (11.1)	.056
BMI, kg/m^2, mean (SD)	28.1 (4.4)	26.5 (4.0)	.094
Fat mass, %, mean (SD)	32.6 (7.8)	29.9 (6.7)	.127
FFMI, kg/m^2, mean (SD)	19.1 (2.1)	18.4 (1.9)	.151
Dynamometry, kg, mean (SD)	33.0 (8.7)	31.6 (7.6)	.487
SGA, %			.526
Well nourished	88.2	82.9	
Moderate malnutrition	11.8	17.1	
Severe malnutrition	0	0	
Pathology			
Urological cancer, %	47.1	42.9	.726
Prostate, No.	16	14	
Bladder, No.	0	1	
Gynecological cancer, %	23.5	22.9	.947
Cervical, No.	4	3	
Endometrium, No.	4	5	
Rectal cancer, No. (%)	7 (20.6)	10 (28.6)	.442
Other tumors, %	8.8	5.7	.618
Stomach, No.	1	1	
Pancreas, No.	1	0	
Lymphoma, No.	1	0	
Liposarcoma, No.	0	1	
Stage, %			.620
I	7.1	15.6	
II	67.9	43.8	
III	17.9	34.4	
IV	7.1	6.3	
Chemotherapy, %	44.1	37.1	.555
Previous surgery, %	32.4	34.3	.865
Brachytherapy, %	23.5	25.7	.833

BMI, body mass index; FFMI, fat-free mass index; SGA, Subjective Global Assessment.

Acute Radiation Enteritis

More patients developed ARE with GLN than with PLA (55.9% vs 22.0%; *P* = .002), with an HR of developing enteritis among the former group of 1.59 (95% confidence interval [CI], 0.62–4.05). The median cumulative dose of radiation to develop diarrhea was 21.6 Gy for GLN and 25 Gy for PLA, but there was no significant difference between the survival curves, as shown in Figure 2. No differences were found in the number of stools per day between GLN and PLA at visit 1 (Md [IQR] = 1.0 [1.0] vs 1.0 [0.8]; *P* = .918) and visit 3 (Md [IQR] = 2.0 [2.0] vs 2.0 [2.0]; *P* = .513). At visit 2, stool frequency was higher with GLN (Md [IQR] = 2.0 [3.5] vs 1.0 [0.8]; *P* = .037). In the middle of RT, GLN was associated with more patients with soft or liquid stools (51.5% vs 18.8%; *P* = .011), but this difference was not found at either visit 1 or visit 3. The grades of acute enteritis according to the RTOG criteria are shown in Table 2. Loperamide was required in 12.1% of patients with GLN and 5.7% with PLA (*P* = .352). Two patients in the placebo group and none in the glutamine group interrupted RT because of diarrhea.

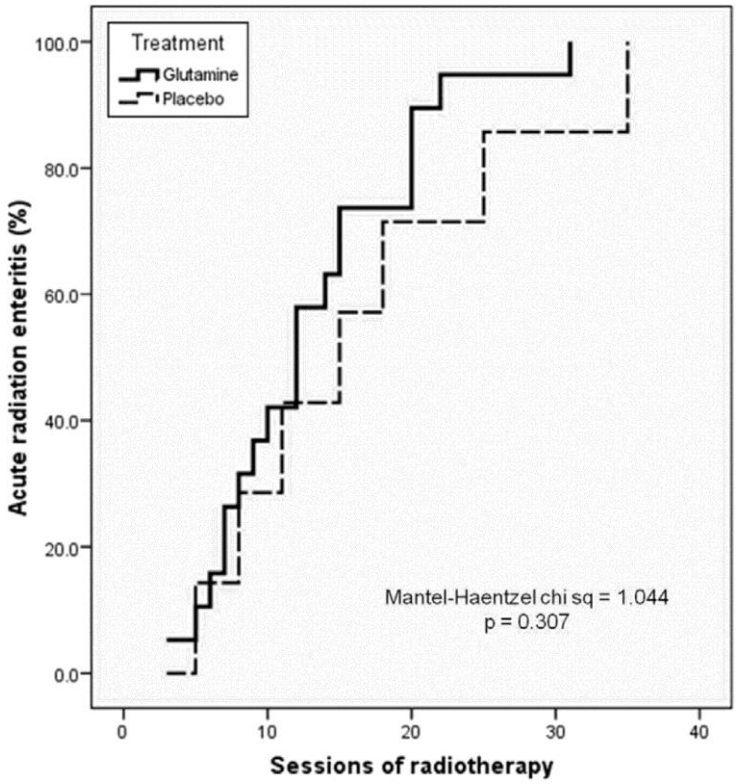

Figure 2. Appearance of acute radiation enteritis according to the intervention. Kaplan-Meier curves represent the new cases of acute radiation enteritis during the radiotherapy. The vertical axis represents the cumulative incidence of intestinal toxicity. The continuous line represents patients receiving glutamine and the dashed line those receiving placebo.

Table 2. Severity of Enteritis According to RTOG Criteria.

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	*P* Value
Visit 2, %						
Glutamine	55.9	23.5	20.6	0	0	.001
Placebo	93.9	6.1	0	0	0	
Visit 3, %						
Glutamine	69.7	9.1	21.2	0	0	.220
Placebo	82.9	8.6	5.7	2.9	0	

RTOG, Radiation Therapy Oncology Group. Statistical analysis performed with analysis of variance.

Among patients with urological neoplasms, ARE was more frequent with GLN than with PLA (50.0% vs 6.7%; P = .008), but there were no significant differences in those with gynecological (75.0% vs 37.5%; P = .131) or rectal cancer (71.4% vs 30.0%; P = .092). After adjusting for different variables (sex, age, chemotherapy, brachytherapy, type of cancer, intervention, body mass index [BMI]) with multivariate analysis, only 3 factors were independently associated with ARE: treatment with glutamine (odds ratio [OR], 7.4; 95% CI, 1.8–30.9), gynecological cancer (OR, 20.0; 95% CI, 2.8–143.9), and rectal neoplasm (OR, 7.9; 95% CI, 1.5–41.5). There were no differences in ARE depending on the dose of glutamine per kilogram of weight.

Citrulline

Before RT, both groups had similar citrulline concentrations (GLN, 30.0 [16.5] mmol/L vs PLA, 37.4 [23.4] mmol/L; *P* = .172), and there was no difference at the end of study (GLN, 26.31 [10.29] mmol/L vs PLA, 27.69 [12.31] mmol/L; *P* = .639). When the overall group of patients was analyzed, PLA was associated with a significant reduction of citrulline compared with baseline (*P* = .040), but it remained unchanged in the glutamine group (*P* = .326). Patients treated with glutamine who developed ARE tended to present lower concentrations of citrulline than those without diarrhea (Figure 3), and there were no differences in PLA. A similar percentage of patients in both groups presented with a citrulline level <20 mmol/L (GLN, 24.14% vs PLA, 25.0%; *P* = .938).

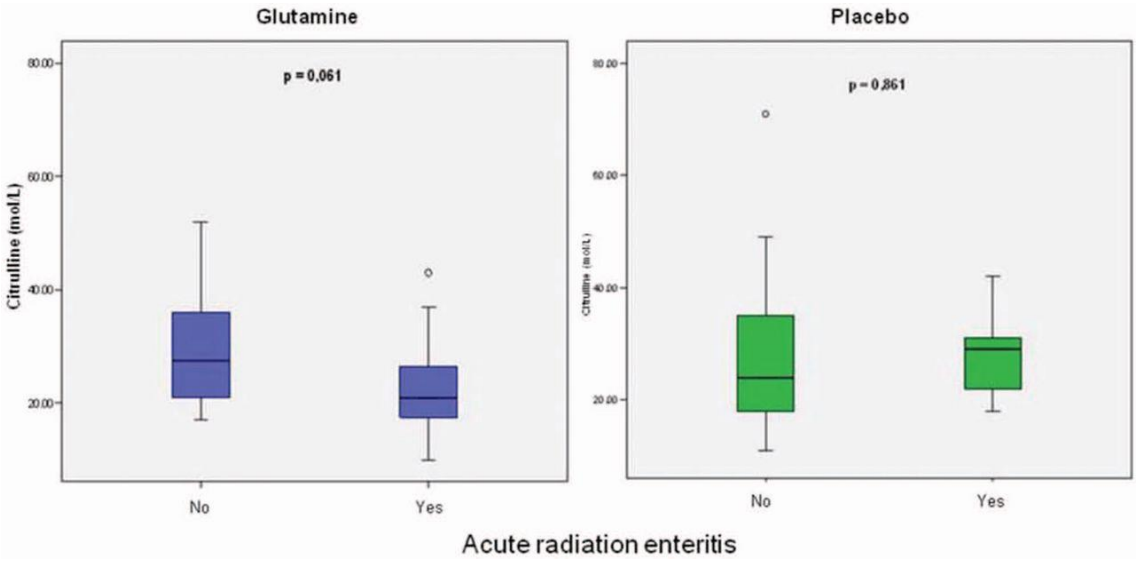

Figure 3. Plasma citrulline concentration after radiotherapy, according to the appearance of acute radiation enteritis. The central line of the box plot represents the median and the lower and upper borders the first and third quartiles, respectively. Patients receiving glutamine who developed intestinal toxicity tended to present with reduced concentrations of citrulline, but this amino acid remained clearly unchanged among those receiving placebo.

Calprotectin

The number of patients with elevated calprotectin before RT differed significantly depending on the underlying disease: rectal cancer (69.2%), prostate cancer (52.0%), and gynecological cancer (7.7%) (*P* = .019). There were no differences in calprotectin concentrations between groups either before (GLN Md [IQR] = 55.0 [134.6] mg/kg, PLA Md [IQR] = 43.4 [105.4] mg/kg; *P* = .949) or after RT (GLN Md [IQR] = 57.9 [85.8] mg/kg, PLA Md [IQR] = 54.0 [57.0] mg/kg; *P* = .182). However, there was an increase in the number of patients with a detectable concentration of fecal calprotectin in GLN during RT (visit 1, 55.2%; visit 3, 83.9%; *P* = .032) that was not observed in PLA (visit 1, 69.0%; visit 3, 66.7%; *P* > .05). In the overall group, there was no difference in the number of patients with calprotectin >50 mg/kg after RT (GLN, 58.1%; PLA, 54.6%; *P* = .777), but when only the patients who developed ARE were analyzed, high calprotectin concentrations were more frequently found in GLN than in PLA (70.0% vs 16.7%; *P* = .022).

Systemic Inflammation

Initial ferritin concentrations were significantly higher among patients with prostate tumors than in patients with other neoplasms (318.7 [435.6] ng/mL vs 98.1 [166.8] ng/mL; *P* < .001), and this difference persisted at visit 2 (337.7 [452.5] ng/mL vs 110.1 [166.1] ng/mL; *P* < .001) and at visit 3 (318.1 [432.7]

ng/mL vs 121.0 [432.7] ng/mL; *P* = .001). This difference remained after adjusting for other variables such as age, sex, body composition, nutrition status, and treatment with chemotherapy or surgery. There were no differences in CRP levels among the different neoplasms at any study visit. In both groups, there was a significant increase in ferritin values at the end of the RT, but not in the concentration of CRP (Figure 4). There were no differences between the 2 groups in the values of these parameters at any visit.

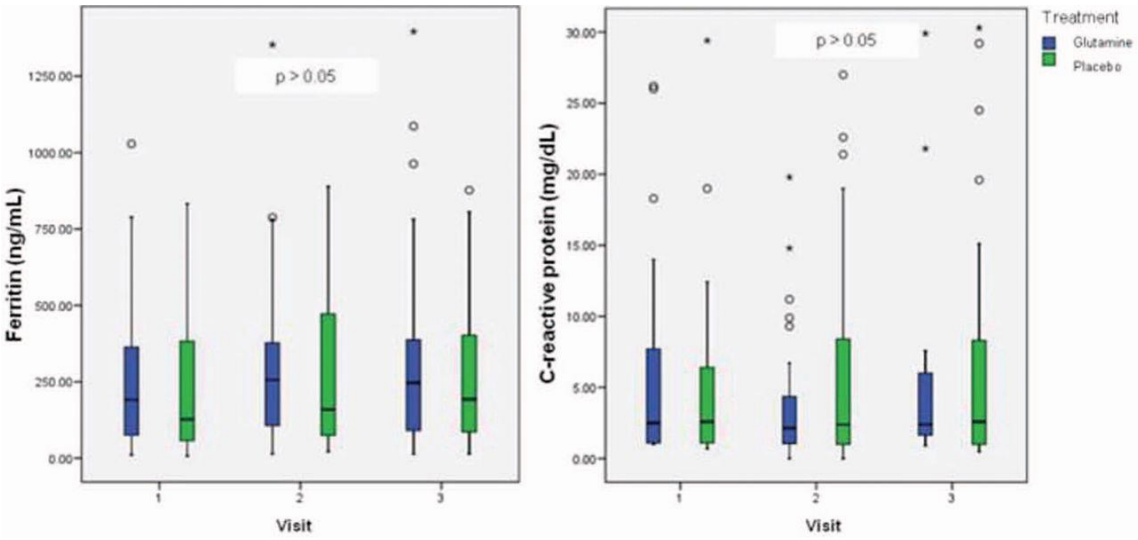

Figure 4. Evolution of systemic inflammatory parameters. Box plot represents the evolution of inflammatory parameters during the follow-up. Ferritin concentrations rose in the both arms of the study during the radiotherapy, but C-reactive protein did not change. There were no differences between the arms of the trial.

Nutrition Status

According to SGA, 10 cases (14.4%) of moderate malnutrition were found before RT, most of them among patients with rectal (4/10) and gynecological tumors (3/10). During follow-up, 8.8% of patients with glutamine and 5.7% with placebo developed malnutrition (*P* = .618). Both groups maintained a stable weight, BMI, FM, and FFMI during follow-up, and the percentage of patients with significant weight loss (>5%) was low in both groups: 3.0% in GLN and 5.7% in PLA (*P* = .590). The evolution of nutrition parameters and macronutrient intake is summarized in Table 3. Both intervention groups consumed a similar amount of micronutrients with antioxidant function during RT (vitamins A, E, and C; selenium; and zinc; data not shown).

Table 3. Evolution of Nutrition Parameters and Dietary Intake.

	Glutamine, Mean (SD)	Placebo, Mean (SD)	*P* Value
Anthropometry			
Weight, kg			
Visit 1	74.8 (13.7)	68.9 (11.1)	.056
Visit 2	75.1 (13.9)	68.6 (11.3)	.041
Visit 3	74.9 (14.3)	68.9 (11.7)	.063
BMI, kg/m^2			
Visit 1	28.1 (4.4)	26.5 (4.0)	.094
Visit 2	28.3 (4.4)	26.2 (3.9)	.046
Visit 3	28.3 (4.5)	26.4 (4.1)	.082
FM, %			
Visit 1	32.6 (7.8)	29.9 (6.7)	.127
Visit 2	31.7 (6.3)	30.0 (7.1)	.288
Visit 3	31.4 (6.7)	28.5 (7.3)[a]	.093
FFMI, kg/m^2			
Visit 1	19.1 (2.1)	18.4 (1.9)	.151
Visit 2	19.1 (2.1)	18.2 (2.0)	.073
Visit 3	19.2 (2.1)	18.7 (2.2)[a]	.427
Weight loss, %			
Before RT	–0.44 (4.88)	–1.30 (4.45)	.444
Visit 2	0.42 (2.42)	–0.54 (1.93)	.081
Visit 3	0.44 (3.26)	–0.14 (3.00)	.452
Dynamometry, kg			
Visit 1	33.0 (8.7)	31.6 (7.6)	.487
Visit 2	32.8 (8.7)	31.2 (7.5)	.421
Visit 3	31.7 (8.0)	31.2 (6.9)	.791
Analysis			
Serum albumin, g/dL			
Visit 1	4.5 (0.3)	4.3 (0.3)	.015
Visit 2	4.4 (0.3)	4.3 (0.3)	.134
Visit 3	4.3 (0.3)[a]	4.3 (0.3)	.411
Prealbumin, mg/dL			
Visit 1	25.1 (5.6)	24.4 (5.5)	.592
Visit 2	24.9 (5.9)	24.8 (6.1)	.964
Visit 3	24.5 (5.4)[a]	25.0 (5.7)	.738
RBP, mg/dL			
Visit 1	4.2 (1.5)	4.3 (1.4)	.683
Visit 2	4.4 (1.5)	4.5 (1.5)	.785
Visit 3	4.4 (1.4)	4.4 (1.2)	.594
Cholesterol, mg/dL			
Visit 1	207.4 (40.2)	200.3 (30.8)	.427
Visit 2	196.1 (36.1)[a]	191.5 (35.5)	.615
Visit 3	197.6 (40.9)	196.7 (32.2)	.920
Dietary intake			
Energy, kcal			
Visit 1	2093.3 (425.1)	2082.9 (555.4)	.932
Visit 2	2170.6 (503.6)	2163.8 (623.4)	.962
Visit 3	2235.8 (589.2)	2206.4 (621.6)	.843
Carbohydrates, %			
Visit 1	44.5 (5.9)	47.0 (7.0)	.120
Visit 2	45.4 (6.5)	46.1 (5.3)	.641
Visit 3	45.4 (7.0)	45.6 (5.2)	.882
Protein, %			
Visit 1	17.6 (2.3)	16.8 (2.6)	.220
Visit 2	17.8 (2.5)	17.5 (2.4)	.691
Visit 3	17.1 (2.6)	17.0 (2.8)	.925
Fat, %			
Visit 1	37.9 (6.2)	36.2 (6.6)	.261
Visit 2	36.8 (6.6)	36.8 (5.6)	.992
Visit 3	37.5 (7.2)	37.4 (4.7)	.932
Fiber, g/1000 kcal			
Visit 1	8.8 (2.8)	8.6 (3.0)	.787
Visit 2	9.4 (2.6)	8.7 (2.6)	.329
Visit 3	9.2 (2.4)	8.8 (3.0)	.527

BMI, body mass index; FM, fat mass; FFMI, fat-free mass index; RBP, retinol binding protein; RT, radiotherapy.
[a]$P < .05$ compared with visits 1 and 2.

Adverse Effects

Four patients with placebo had vomiting that forced them to stop treatment, but glutamine was well tolerated in all patients. Compliance was assessed counting the remaining envelopes after the finish of radiotherapy, and all patients except those not tolerating the placebo correctly took the provided treatment. The glomerular filtration rate was stable in both groups during follow-up. Patients did not have high PSA values at the end of RT, and the same percentage had high CEA concentrations (GLN, 14.3% vs PLA, 12.5%; $P = .919$). Other adverse effects are summarized in Table 4.

Table 4. Adverse Effects During the Study.

Adverse Effect	Glutamine, No. (%)	Placebo, No. (%)	*P* Value
GFR <60 mL/min	3 (8.8)	4 (11.4)	.689
AST >100 IU/L	0	0	
ALT >100 IU/L	0	1 (2.9)	.306
Anemia	2 (5.9)	1 (2.9)	.554
Leucopenia	3 (8.8)	6 (17.1)	.279
Lymphopenia	26 (76.5)	26 (74.3)	.635
Neutropenia	2 (5.9)	2 (5.7)	1.000
Thrombocytopenia	0	2 (5.7)	.157

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GFR, glomerular filtration rate; IU, International Units. Hematology toxicity was defined following the Common Toxicity Criteria of the National Cancer Institute. Anemia: hemoglobin <10 g/dL; leucopenia: leukocytes <3000/mm^3; lymphopenia: lymphocytes <1000/mm^3; neutropenia: <1500/mm^3; thrombocytopenia: platelets <75,000/mm^3.

Discussion

Some nutrients, including glutamine, have been proposed for modifying the therapeutic window by enhancing the cytotoxic action on the tumor or by preserving healthy tissue. Diarrhea is one of the most common side effects of cancer therapy, affecting up to 50% of patients with chemotherapy and 30% with pelvic RT. In this context, a randomized, controlled, double-blind trial was designed to test the effectiveness of glutamine in the prevention of radiation enteritis. The results show that glutamine was independently associated with the development of ARE.

Only 2 studies suggesting some benefit of glutamine during pelvic RT have been published and only in abstract form. The study by Richards et al[6] included patients with prostate cancer who received either glutamine (21 g/d) or placebo. The former was associated with less tissue damage in the rectum, but there was no difference in stool frequency. In the second study, which included mostly patients with gynecological tumors, glutamine (30 g/d) was associated with a lower incidence of ARE, but this study was neither blinded nor controlled.[9] A third study included a single cohort of patients with gynecological or rectal cancer receiving 30 g of oral glutamine, in which ARE occurred in 73% of patients, a percentage similar to that observed in patients with the same tumors who received glutamine in our trial.[11] Only 2 RCTs have been fully published. The study by Kozelsky et al[7] included patients with pelvic cancer, and there were no differences in the development of acute toxicity. The second RCT included patients with rectal cancer who received glutamine (30 g) or placebo. In the glutamine group, more patients developed ARE than with placebo, although this difference was not significant.[8] Among the weaknesses of this study was that an intention-to-treat analysis was not performed. The sample size was smaller than that of our trial, and this may have influenced the statistical significance.

Arginine supplementation in rats receiving abdominal RT has been related to greater colonic damage, possibly caused by nitric oxide (NO) overproduction.[12] NO plays a dual role in the gut by promoting the maintenance of barrier function and compromising the viability of intestinal cells when generated in excessive amounts. The addition of glutamine to intestinal cells in vitro does not favor the formation of NO, but glutamine could act indirectly by the glutamine-citrulline-arginine metabolic pathway. Macrophages cultured in glutamine-enriched medium and stimulated with lipopolysaccharide are able to produce NO even in the absence of arginine, suggesting that a stimulus may be necessary for glutamine to

enhance the production of NO.[13] This mechanism may explain why glutamine-related adverse effects have not been reported when radiation therapy is administered in body areas (head and neck, esophagus) where there is no local production of citrulline, which can serve as a substrate for NO synthesis. In addition, NO may play a dual role as a radiosensitizer or radioprotector. The former is mediated through its antioxidant properties when it is generated in low concentrations and the latter in combination with the superoxide anion.[14] These actions of NO are dose dependent, and it is necessary to achieve a minimum threshold to produce toxicity. Glutamine in this context may be a substrate that would favor excessive production by the mechanisms described above, explaining why the study providing 21 g of the amino acid had no adverse effects.[6,8] In addition, glutamine might behave at certain doses as a pro-oxidant agent. Glutamine at doses of 0.15 g/kg, administered to women with breast cancer treated with chemotherapy, failed to increase glutathione levels in healthy breast tissue and plasma.[15] Shinozaki et al[16] observed in rats with chemically induced colitis that a diet containing 12% glutamine was associated with lower intestinal inflammation than a diet free of this amino acid, but a diet that provided 24% glutamine produced more inflammation.

The concentration of calprotectin increases as a result of treatment with RT. In this study, there was an increase in the number of patients who had detectable levels of calprotectin in the glutamine group, and among patients who developed enteritis, the frequency of elevated calprotectin was higher in those assigned to glutamine. These findings suggest the development of intestinal inflammation associated with glutamine treatment. The final concentrations of calprotectin did not differ between groups. It has been described that calprotectin reaches a maximum value after 5 weeks of RT and subsequently decreases gradually. So, it is possible that when the final sample was obtained, the calprotectin concentrations were already in decline or that liquid stools could have diluted the concentrations of calprotectin. Rectal and prostate cancers were more frequently associated with elevated calprotectin before RT. It has been reported that carcinomas and colon adenomas express calprotectin. However, it is striking that more than half of patients with prostate cancer showed intestinal inflammation, as well as higher ferritin levels. The patients with prostate cancer enrolled in this study were undergoing androgen deprivation therapy with GnRH analogues. In mice, castration has been associated with increased intestinal inflammatory response secondary to the administration of radiation.[17] Human data on the immune status of men with hypogonadism are scarce. A case-control study reported an exacerbation of the specific immune response after different injuries, inversely proportional to the concentrations of circulating testosterone.[18]

Systemic inflammatory response was similar in both arms. In a study by Kozjek et al,[8] a significant elevation of interleukin-6 was associated with glutamine. The role of glutamine in the regulation of cytokine production seems to depend on factors such as dose as well as the noxious stimulus. In the healthy human gut, glutamine administration decreases the production of proinflammatory cytokines.[19] Nevertheless, the administration of glutamine above physiological concentrations can directly stimulate the release of TNF-α and also increases the production of NF-κB and interleukins in macrophages stimulated by lipopolysaccharide.[20]

Citrulline values decrease with the administration of radiation proportionally to the dose received by the intestine, especially from the third week of treatment.[21] In this trial, the concentration of citrulline significantly decreased in the placebo group after radiation therapy but remained stable in patients receiving glutamine. These results could indicate that there was a better conservation of villous mass with the administration of glutamine, although these patients presented with ARE and intestinal inflammation more frequently. However, some studies have shown a poor correlation between the clinical severity of ARE and citrulline.[22]

The main strength of the present trial includes its methodology, as randomized, controlled, double-blind trials on the use of glutamine in abdominal/pelvic radiation therapy are scarce. However, some limitations should be noted. First, this study was performed in a single center, making it difficult to generalize the results given differences in the population or RT techniques. Second, citrulline and calprotectin could not be analyzed in the middle of treatment, when ARE was more frequent and clinically evident. Third, specific markers of inflammation (eg, TNF-α, interleukins) or oxidative stress could not be evaluated. Finally, a larger sample size would have added potency to the study.

In conclusion, the administration of oral glutamine during abdominal or pelvic RT was not useful for the prevention of acute enteritis and could have favored the development of diarrhea. This effect may be specifically related to intestinal exposure to radiation, as it has not been observed in other kinds of diarrhea or in RT over other corporal areas, as well as to the administered dose (30 g). The mechanisms explaining the relationship between gut inflammation, glutamine, and radiation are not clear and should be researched in new studies.

Article Notes

Financial support This research has received grants from Fundación de la Sociedad Española de Endocrinología y Nutrición and Consejería de Sanidad (Junta de Castilla y León, SACYL GRS 326/B/08).

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