Clinical Nutrition 30 (2011) 567–570

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Oral glutamine supplementation during preoperative radiochemotherapy in patients with rectal cancer: A randomised double blinded, placebo controlled pilot study

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ARTICLE INFO

Article history:
Received 26 January 2011
Accepted 5 June 2011

Keywords:
Glutamine
Enteral
Radiochemotherapy
Diarrhoea
Inflammation
Metabolic stress
Preoperative
Rectal cancer

SUMMARY

Background & aims: Enteral glutamine may have protective effects on gut function and reduce metabolic stress in patients receiving radiochemotherapy. The aim of our study was to evaluate its influence in patients with rectal cancer undergoing preoperative radiochemotherapy.
Methods: We performed a randomized double blind, placebo controlled pilot study in 33 patients. 30 g of glutamine, average dose 0.41 g/kg (SD = 0.07) g/kg/day was administered orally in three doses per day for five weeks during preoperative radiochemotherapy of rectal cancer. 30 g of maltodextrin was given as placebo. Body weight was measured and NRS 2002 screening was performed before and after treatment. Bowel function was evaluated by stool consistency and frequency. Plasma levels of inflammatory parameters and hormones were measured.
Results: There was no difference between groups in frequency and severity of diarrhoea during radiochemotherapy ($p = 0.5$ and $p = 0.39$ respectively), insulin levels significantly increased in both groups, IL-6 only in glutamine group.
Conclusion: Results of this small pilot study in rectal cancer patients receiving preoperative radiochemotherapy, showed that ingestion of larger quantities of glutamine given more often as previously reported did not diminish the incidence and severity of diarrhoea and did not affect inflammatory and metabolic activity compared to the placebo treatment with maltodextrin.

1. Introduction

Glutamine is the most abundant amino acid in the body and is used as a fuel for rapidly proliferating cells.[1,2] It is an important factor for immune system, as an energy source for immune cells and a crucial amino acid required for their differentiation and growth.[3,4] It has been shown that glutamine is essential amino acid during periods of prolonged metabolic stress because of the inability of mammals to synthesize glutamine in sufficient amounts.[5]

Malignant diseases and their treatment produce systemic inflammatory response and metabolic stress, and therefore affect the availability of glutamine to immunocytes.[6] The review of Kuhn et al. introduced the hypothesis that some types of tumour cells are able to manipulate host metabolism to cover their needs for glutamine, when it was not available from exogenous sources. They concluded that glutamine deficiency didn't affect tumour growth but it contributed to the development of impaired physiological functions such as disturbed mucosal integrity and diminished immune competence.[7] Studies on transport of amino acids between organs in animals with cancer also suggested that presence of cancer induces gut dysfunction and increases glutamine disposal, leading to disturbances in the integrity of the gut.[8–10] In addition, exposure of abdominal region to ionizing radiation during radiotherapy in itself causes mucosal damage, intestinal inflammation and dysfunction.[11] In animal studies, glutamine supplementation before or after whole body irradiation decreased the severity of both acute and chronic toxic effects of radiation on the

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0261-5614/$ – see front matter
doi:10.1016/j.clnu.2011.06.003

lower intestine.[12,13] Finally, chemotherapy produces inflammatory changes in the intestinal mucosa including increased gut permeability.[8] Oral glutamine can counteract these effects. This effect was demonstrated in patients with colorectal and breast cancer treated with chemotherapy.[14–16]

As glutamine is metabolized in rapidly proliferating cells in the gut, the feeding route would influence its availability and effect.[17] It is possible that enteral glutamine administered to patients receiving radiochemotherapy for colorectal diseases reaches the intestine preferentially than parenteral glutamine. Even if ESPEN guidelines on enteral nutrition for non-surgical oncology did not find enough evidence to recommend glutamine supplementation to these patients, others do recommend its use in the patients receiving chemotherapy.[18,19] However, studies greatly differ in the amount and frequency of enteral dose of glutamine, which may explain discrepancy of results of different trials.

We therefore hypothesized that increased daily dose and frequency of enteral glutamine will decrease intestinal toxicity of radiochemotherapy, the inflammatory response, and metabolic disturbances in rectal cancer patients receiving preoperative radiochemotherapy. We designed a double blind, placebo controlled study with a primary endpoint of change in intestinal function indicated by diarhoea. Secondary endpoint was change in radiochemotherapy induced inflammatory response and metabolic disturbances.

2. Patients and methods

Sixty rectal cancer patients receiving preoperative radiochemotherapy were planed to be included in the study. The closed envelopes were randomly allocated in a 1:1 ratio to control or glutamine group using computer generated randomisation. We started this pilot study in May 2008 and performed an interim analysis in March 2009. At this time 41 patients (24 men and 17 women) were enrolled and the drop out was 8 patients (4 men, 4 women). They were excluded from the final analysis due to failure to comply with ingestion of the enteral supplements.

Study was done at the Institute of Oncology in Ljubljana. Patients were enrolled by attending physicians during their first visit to the radiotherapy department and instructed about the study by clinical dietitian. Computerized randomisation was made at department for clinical studies. Random numbers were allocated to sequentially numbered containers for glutamine and placebo. The study was double blinded for medical personal and patients. Pharmacist was the only person who knew which numbers were allocated to which treatment group.

Glutamine was administered orally (30 g/day, divided in three doses) at the start of radiochemotherapy and for the subsequent 5 weeks of standard preoperative treatment of rectal cancer with radiochemotherapy. The placebo group received 30 g of maltodextrin as oral supplement, divided in three doses. Both intervention supplements were in powder form, indistinguishable from each other, both were dissolved in glass of cold water. Glutamine powder was provided by Peeroton Warenhandelsgesellschaft m.b.H. (Austria**).** Maltodextrin powder was provided by Nutricia, Cuijk (Netherlands). Patients were asked to take the supplement before their meals.

A total irradiation dose of 45 Gy was administered to the pelvis in 1.8 Gy daily fractions over 5 weeks and 5.4 Gy as a boost to the primary tumour. Chemotherapy was administered concomitantly with radiotherapy and consisted of capecitabine given orally at a daily dose of 1650 mg/m^2, divided in two equal doses given 12 h apart. One of the doses was taken 1 h prior to irradiation. Chemotherapy started on the first day of radiotherapy and finished on the last day of radiotherapy (including weekends).

Table 1
Criteria for diarrhoea (patient − reported symptoms).

Grade of diarrhoea	Criteria
Frequency	
1	No liquid stool or increased stool frequency
2	Liquid stool 1–2 times per day (mild)
3	Liquid stool 3–4 times per day (moderate)
4	Liquid stool 5–7 times per day (severe)
5	Liquid stool > 7 times daily (very severe)

Before and after treatment body weight was measured and Nutritional Risk Screening (NRS) 2002 was performed in each patient.[20]

During the study, we assessed radiochemotherapy toxicity by evaluating bowel function. We recorded incidence and severity of diarrhoea using an adapted questionnaire as recommended by the National Cancer Institute (USA) (Table 1). Patients answered this questionnaire at the end of the 5-weeks period.

Blood samples were taken before and at the end of treatment to analyse inflammatory parameters: complete blood count, interleukin 6 (IL-6) and lipopolysacharide binding protein (LBP) and metabolic activity including cortisol, insulin and testosterone. Serum samples were analysed on Modular Analytics SWE (Roche Diagnostics), fully automated, software controlled system for clinical chemistry. Serum concentrations of IL-6 and LBP were measured using Immulite chemiluminiscence immunoassay system (Siemens). Complete blood count, including white blood cell and differential count, was performed on LH 750 haematology analyser (Beckmam Coulter).

Statistics: The symptoms of diarrhoea were reported on a categorical scale, other variables were numerical. Numerical data were presented as means and SD, categorical data as absolute numbers with relative frequencies. All numerical data were initially tested for normality using the Kolomogorov–Smirnov test. For comparison of results between glutamine and placebo groups Student *t*-test and Chi-square tests were applied. The SPSS version 12.0 for Windows was used for statistical analysis.

The study was approved by the hospital medical ethics committee and by the national ethics committee. All patients gave written informed consent before enrolment.

3. Results

Final analysis was carried out in 33 patients out of 41 studied. Eight patients dropped out (4 glutamine, 4 placebo group). Both groups of patients were similar in age, weight and nutritional status

Table 2
Patients' data.

	Glutamine Mean ± SD	Placebo Mean ± SD	*P*-value
Age	60.5 ± 14.2	63.6 ± 10.12	0.48
Weight loss in 3 months before (kg)	3.00 ± 3.2	2.24 ± 2.8	0.47
Patient's body weight at the start (kg)	73.86 ± 12.9	73.76 ± 16.6	0.98
NRS 2002 at the start	1.14 ± 1.03	1.58 ± 1.43	0.34
TN clinical stage (%):			
T3N0	38.5	16.7	
T3N1	30.8	44.4	
T3N2	15.4	27.8	
T2N0	7.7		
T2N2		5.6	
T4N0	7.7		
T4N2		5.6	
WHO performace status (%)			
0	76.9	77.8	
1	23.1	22.2	

Table 3
Frequency of diarrhoe.

Diarrhoe intensity	Glutamin	Placebo
No diarrhoe	5	8
Mild diarrhoe	4	2
Moderate	4	4
Severe	0	2
Very severe	1	1

at the beginning of the treatment. NRS 2002 at the beginning of treatment and weight loss in three months before treatment was not significantly different between both groups (Table 2).

No unwanted effects of the intervention were reported. In the glutamine group all patients received more than 0.2 g of glutamine/kg/day, on average 0.41 g/kg (SD +/− 0.07) g/kg/day 6 patients received 0.2–0.4 g/kg/day and 8 patients more than 0.4 g/kg/day. No differences in occurrence of diarrhoea were found in these subgroups. In the glutamine group 9 patients had diarrhoea, 4 mild, 4 moderate, and 1 very severe diarrhoea. In the placebo group 10 patients had diarrhoea: 2 mild, 4 moderate, 3 severe and 1 very severe diarrhoea. Fourteen patients had no diarrhoea, 5 in the glutamine and 9 in the control group. Data from 3 patients were not reliable (Table 3). No significant differences were found between the groups in the incidence of diarrhoea ($p = 0.50$; chi-square test) and in its intensity ($p = 0.39$; chi-square test).

Insulin blood concentrations were significantly higher at the end of treatment compared to pre-treatment levels in the glutamine group ($p = 0.01$) and in the placebo group ($p = 0.03$). IL-6 rose to a similar extent in both groups after 5 weeks of radiochemotherapy, but significance was reached only in the glutamine group ($p = 0.007$) due to a smaller standard deviation at the start of treatment. White cells and lymphocyte counts decreased significantly in both groups when compared to pre-treatment levels. These changes were not significantly different between both groups (Table 4).

4. Discussion

Preoperative radiochemotherapy is currently the standard therapeutic approach in patients with rectal cancer.[21] However, it induces a systemic and local inflammatory response and may be associated with potential glutamine deficiency, thus contributing to intestinal dysfunction.[8,11] Enteral glutamine supplementation in animals has been shown to diminish gastrointestinal toxicity during radiotherapy, but clinical studies with enteral glutamine supplementation have produced inconsistent results.[8,11,22–25]

In our double blind randomised pilot study, who is limited by small size of pilot group and potential effects of maltodextrine, no benefit of enteral glutamine supplementation was found with respect to the incidence and severity of diarrhoea. Our results were similar to those reported by Kozelsky et al., who also found no beneficial effect of glutamine during pelvic radiotherapy. In view of recommended doses of glutamine supplementation, their result was expected because they used a small dose of only 8 g of glutamine per day for 2 weeks.[22,26] We administered larger dosages of oral glutamine divided in three daily doses in an attempt to get better availability of glutamine. Bozzeti studied the effect of the same amount glutamine and same placebo supplement on doxifluridine induced diarrhoea in breast cancer patients. However, in his study glutamine was given for only 8 days in the intervals between chemotherapy which lasted 4 days. He did not find differences in incidence or severity of diarrhoea.[25] Also in our study no improvement in incidence and severity of diarrhoea, was found, despite supplementing glutamine for 5 weeks in dosage of 30 g per day.

Opposite, Huang et al. administered the same quantity of glutamine as we did, and found that the incidence of mucositis decreased.[23] Yoshida Shogo's group also used 30 g of glutamine for a shorter period of 28 days in 13 patients with advanced oesophageal cancer undergoing radiochemotherapy. At this dose they found an improved gut mucosal integrity and preservation of plasma lymphocyte numbers.[24] Unfortunately they did not report the incidence of diarrhoea in these patients. In a review of available studies, it was concluded that the supplementation with glutamine significantly shortened the duration of diarrhoea and lowered its frequency in chemotherapy patients.[27] Oral glutamine was also shown to favourably affect chemotherapy induced mucositis.[6] It is difficult to explain discrepancy between these results and the lack of benefit in our study. It is still possible that lack of a benefit on systemic inflammation and diarrhoea was because of insufficient amounts of glutamine which reahed the circulation to achieve a systemic influence despite the high enteral dosages given for a long time.[28,29]

As far as we know, no studies have until now addressed the effect of oral glutamine supplementation on diarrhoea in preoperative rectal cancer treated with radiochemotherapy. Similarly, we are not aware of other studies in patients assessing the inflammatory and hormonal response to oral glutamine administration in this group of cancer patients. Our small pilot study showed no significant difference in inflammatory response and metabolic activity during radiochemotherapy. The decrease in white cell counts are very likely caused by chemotherapy related suppression of white cell formation, possibly in conjunction with the long term immunosuppressive effect of inflammatory activity induced by radiotherapy. However, this study has limitation regarding low power which leads to an unclear statistical comparison, but we wanted to give valuable information for further clinical research and some orientation for future more extensive, probable multicentric study.

Table 4
Hormonal and inflammatory response before and at the end of radiochemotherapy treatment in glutamine and placebo group.

	Glutamine $n = 14$			Placebo $n = 19$			*P*-value of difference before-after treatment[a]
	Before treatment Mean ± SD	After treatment Mean ± SD	*P*-value	Before treatment Mean ± SD	Mean ± SD	*P*-value	
Testosteron nmol/l	6.4 ± 6.2	4.8 ± 5.3	0.14	7.1 ± 6.7	7.0 ± 6.8	0.88	0.26
Cortisol nmol/l	381 ± 200	294 ± 89	0.14	405 ± 141	373 ± 152	0.53	0.40
Insulin U/l	18.5 ± 16.7	46.5 ± 42.7	0.01	22.7 ± 30.8	38.6 ± 38.1	0.03	0.31
IL-6 pg/ml	3.0 ± 1.7	6.6 ± 5.7	0.007	5.1 ± 3.3	8.8 ± 5.6	0.15	0.92
LBP µg/ml	6.7 ± 1.8	7.2 ± 3.7	0.57	7.3 ± 2.4	9.3 ± 4.4	0.11	0.35
CRP mg/l	2.3 ± 2.2	5.7 ± 11.1	0.21	9.8 ± 7.8	16.3 ± 21.2	0.22	0.57
Leucocytes 10E9/l	7.9 ± 1.8	4.6 ± 1.2	0.0000004	7.7 ± 2.8	4.8 ± 1.4	0.00003	0.65
Lymphocytes %	26.6 ± 6	11.2 ± 4	0.0000009	21.2 ± 7.3	10.2 ± 5.2	0.0000006	0.14
Blood glucose mmol/l	5.2 ± 1.2	6.1 ± 0.8	0.063	6.2 ± 2.1	7.4 ± 2.2	0.43	0.99
Transferrin µmmol/l	34.8 ± 6.9	34.4 ± 7.1	0.11	35.3 ± 4.7	34.9 ± 4.9	0.75	0.96

[a] Differences between glutamine and placebo group in hormonal and inflammatory response.

Therefore we can only conclude that it is possible that higher doses of enteral glutamine given in multiple daily doses did not influence incidence and severity of diarrhoea in rectal cancer patients undergoing preoperative radiochemotherapy.

Statement of authorship

N.R.K designed the study, analysed the data and wrote the manuscript. L.K. and P.S. contributed to interpretation of the data, the discussion and the writing of the manuscript. D.M.M., I.O., V.V. and F.A. helped to collect data and study design. B.M. analysed the laboratory data. V.Z. was responsible for statistical analysis. All authors read and approved the final manuscript.

Conflicts of interest

The authors have no financial or other relations that could lead to conflict of interest.

Acknowledgements

We thank clinical dietitians who made nutritional assessment, pharmacists in Pharmacy department who prepared glutamine and placebo samples and coworkers in the department of clinical chemistry.

Extra costs for laboratory analysis and nutritional supplements were covered from Research programme P4-0092 Ministry of Science Republic of Slovenia.

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