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Original Paper

Reduction of Chemotherapy-induced Side-effects by Parenteral Glutamine Supplementation in Patients with Metastatic Colorectal Cancer

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In animal studies, glutamine (Gln) reduces chemotherapy-associated mucositis and mucosal atrophy. Therefore, this study examined the protective effects of a parenteral Gln supplementation in patients with metastatic colorectal carcinoma receiving 5-fluorouracil (5-FU)/calcium-folinate (CF) chemotherapy. In a prospective study, a total of 24 patients underwent three courses of 5-FU/CF chemotherapy and were randomised with ($n = 12$) or without ($n = 12$) glycyl-L-glutamine. Effects on gastrointestinal mucosa were assessed by endoscopic examinations and histomorphometric measurements. Clinical side-effects were documented according to the World Health Organisation grading. In the Gln group, a significant reduction in mucositis and ulcerations of the gastric ($P < 0.01$) and duodenal mucosa ($P < 0.05$) was documented after the third course of chemotherapy. In the same group, the villus height/crypt depth ratio was significantly higher after therapy than in the unsupplemented group (1st course $P < 0.01$; 3rd course $P < 0.05$). However, there were no significant differences in the incidence and severity of clinical side-effects. The results suggest that parenteral Gln supplementation protects the gastrointestinal mucosa against 5-FU/CF chemotherapy-induced damage.

Key words: colorectal carcinoma, glutamine, chemotherapy, clinical side-effects, gastrointestinal mucosa
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INTRODUCTION

CHEMOTHERAPEUTIC AGENTS affect not only tumour cells, but also all cells with a high proliferative rate, such as enterocytes [1]. This results in side-effects such as mucositis and diarrhoea. Chemotherapy-induced toxicity to normal tissues causes dose reduction with a negative influence on response [2].

The non-essential amino acid glutamine (Gln) is an important nutrient of the gastrointestinal tract and is necessary to protect the structure and function of the gut [3, 4]. In catabolic diseases such as cancer, gut Gln uptake increases. Simultaneously, the muscle Gln stores are depleted in cases of concurrent malnutrition. In these situations a sufficient Gln intake becomes necessary to restore gut integrity [5]. Toxicity of chemotherapy can lead to reduced food intake and Gln deficiency. Animal studies have shown that Gln supplementation reduces chemotherapy-induced mucositis and mucosal atrophy, improves nutritional status and also prolongs survival time [6, 7].

This prospective, controlled and randomised study, investigated the effects of parenteral Gln supplementation on 5-fluorouracil (5-FU)/calcium-folinate (CF) chemotherapy-associated alterations of the gastrointestinal mucosa in humans.

PATIENTS AND METHODS

Written informed consent was obtained from 24 patients with metastatic colorectal carcinoma, who were randomly allocated to two groups. The Gln group ($n = 12$) received parenteral Gln supplementation in combination with 5-FU/CF chemotherapy, while the controls ($n = 12$) received

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chemotherapeutic treatment without Gln supplementation. The patients were observed over three courses of cytostatic therapy. 5-FU (550 mg/m^2/day) was given by continuous 5-day infusion and CF (170 mg/m^2/day) as an intravenous (i.v.) bolus injection on each of these days. The chemotherapy was repeated every 4 weeks. In the Gln group ($n = 12$), Gln was administered as glycyl-L-glutamine (GlyGln) i.v. over 8 h in a 10% solution. Gln was supplemented 1 day before the beginning and during chemotherapy at a dosage of 0.4 g/kg body weight per day of GlyGln (14–22 g Gln/day).

Before and after the first and third chemotherapy courses, the effectiveness of the Gln supplementation was monitored by gastroscopy and duodenoscopy after an overnight fast. The endoscopist did not know to which group the patients belonged. Biopsies of the distal duodenum were taken using the suction biopsy capsule model Baumgartner/Classen with a cutting head. Specimens obtained were submitted for measurement of intracellular Gln concentrations and for histomorphometric examinations. At the same time venous blood samples were taken to determine plasma amino acids, pre-albumin, retinol binding protein (RBP) and routine biochemical parameters. Blood samples were also taken before and after the second chemotherapy course.

Food intake during chemotherapy was recorded using the precise weighing method [8] and nutrient intake was calculated using the software program Prodi 3.2 Plus (Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany) on the basis of the German food composition table (Bundeslebensmittelschlüssel II.1).

All chemotherapy-induced side-effects were documented during the whole study course and graded according to World Health Organisation (WHO) criteria [9]. In addition, anal mucositis was assessed by observation and a classification was used based on the grading of oral mucositis. Gastrointestinal mucositis was documented and analysed 1 day before and 1 day after each chemotherapy course to examine the effects of Gln supplementation. The findings were assessed using a scale from zero to five points (no abnormalities, 0 points; mucositis, 1–2 points; ulcerations, 4–5 points). The findings before chemotherapy were compared with the findings after chemotherapy and a score was calculated with the range +4 to −4.

Blood samples for the determination of amino acids, pre-albumin and RBP were kept on ice immediately after collection. After centrifugation (4°C) plasma was stored at − 20°C. Plasma amino acids were analysed by cation-exchange chromatography and proteins by an immunoassay. The method of determination of amino acid concentration in mucosal biopsies has been described in detail previously [10]. Histomorphometric measurements were performed using an ocular with a micrometer (Karl Zeiss, Jena, Germany). Villus and crypt size were measured in at least ten well-orientated villus or crypt columns.

Table 1. *Patient data*

	Gln group ($n = 12$)	Controls ($n = 12$)
Age (mean ± S.D.)	56.1 ± 9.6 years (range = 40–67)	58.4 ± 7.2 years (range = 44–66)
Sex	4 female/8 male	5 female/7 male
Response*		
Partial response	3	4
No change	5	5
Progressive disease	4	3

*Classification according to the guidelines of the World Health Organisation.

All results are expressed as the mean ± S.D. The Wilcoxon Rank Sum test was used for comparison before and after treatment in groups. Group comparisons for statistical significance were performed using the Mann–Whitney *U*-test. *P*-values < 0.05 were considered significant.

The study was approved by the Medical Ethical Committee of the University of Heidelberg.

RESULTS

Both study groups were comparable in age and response to chemotherapy (Table 1). Because of severe side-effects, the chemotherapy dose had to be reduced in two cases in the control group and in one case in the Gln group. Mean body weight, mean plasma pre-albumin concentration and mean plasma RBP concentrations did not differ between the two groups, either before or during the study (Table 2). All of these parameters were in the normal range. During the first and second therapy courses, there were no differences between the groups concerning energy ingestion (first chemotherapy course: Gln group 108.4 kJ/kg body weight/day versus controls 112.5 kJ/kg/day; second chemotherapy course:

Table 2. *Parameters of the nutritional status and plasma glutamine (Gln) concentrations*

	Chemotherapy (CT) course	Before/after CT	n^*	Body weight (kg)	Pre-albumin (mg/100 ml)	RBP (mg/100 ml)	Plasma Gln concentrations (μmol/l)
Gln group	1	Before	12	73.7 ± 8.7	30.7 ± 8.3	5.0 ± 1.6	609.3 ± 124.0
		After	12	73.3 ± 8.5	27.3 ± 8.7	4.6 ± 1.6	597.1 ± 80.8
	2	Before	12	73.9 ± 8.6	30.5 ± 10.6	5.3 ± 2.4	567.3 ± 149.5
		After	12	73.0 ± 8.4	28.0 ± 12.9	4.7 ± 2.3	582.0 ± 120.0
	3	Before	12	74.2 ± 8.4	29.1 ± 8.7	4.9 ± 1.9	546.7 ± 102.8
		After	11	73.5 ± 8.4	26.2 ± 12.0	4.3 ± 2.4	548.7 ± 65.6
Controls	1	Before	12	69.8 ± 14.8	27.6 ± 6.0	4.2 ± 1.7	597.5 ± 144.4
		After	12	68.9 ± 14.2	27.9 ± 10.3	4.6 ± 2.3	571.4 ± 132.5
	2	Before	12	70.1 ± 14.4	29.8 ± 8.0	5.2 ± 1.9	578.7 ± 83.3
		After	11	69.5 ± 15.0	28.6 ± 7.5	5.0 ± 1.8	596.3 ± 94.8
	3	Before	11	69.5 ± 15.4)	31.2 ± 9.3	5.4 ± 2.7	539.0 ± 74.7
		After	10	69.5 ± 15.4	30.0 ± 9.9	5.3 ± 3.0	556.7 ± 71.8

Data are expressed as means ± S.D. *Data from patients with reduced doses of chemotherapeutic agents were excluded. RBP, retinol binding protein.

Table 3. Ratio of villus height to crypt depth

	First chemotherapy course				Third chemotherapy course			
	n	Before	After	*P*	*n*	Before	After	*P*
Gln group	7	3.4 ± 0.31	3.8 ± 0.26	0.06	8	3.2 ± 0.58	3.6 ± 0.49	0.07
Controls	7	3.2 ± 0.63	3.0 ± 0.57	0.46	9	3.1 ± 0.41	3.0 ± 0.65	0.471

Data are expressed as means ± S.D. Gln, glutamine.

Gln group 101.8 kJ/kg/day versus controls 114.1 kJ/kg/day) and protein ingestion (first chemotherapy course: Gln group 0.91 g/kg/day versus controls 0.94 g/kg/day; second chemotherapy course: Gln group 0.82 g/kg/day versus controls 0.96 g/kg/day). During the third course of therapy the controls had a significantly higher mean energy (Gln group 89.8 kJ/kg/day versus controls 110.4 kJ/kg/day, $P = 0.01$) and protein intake than the Gln group (Gln group 0.76 g/kg/day versus controls 0.96 g/kg/day, $P < 0.05$). However, the mean total protein intake in the Gln group (oral plus GlyGln infusion) remained significantly higher than that in the controls (first chemotherapy course: Gln group 1.30 g/kg/day versus controls 0.94 g/kg/day, $P = 0.001$; second chemotherapy course: Gln group 1.21 g/kg/day versus controls 0.96 g/kg/day, $P = 0.01$; third chemotherapy course: Gln group 1.18 g/kg/day versus controls 0.96 g/kg/day, $P < 0.05$). Plasma Gln concentrations were unchanged (Table 2), but the intracellular Gln concentration in the duodenal mucosa of the supplemented group was higher than that in controls after the third course of chemotherapy (Figure 1).

Technical problems with the suction biopsy capsule led to a reduced number of biopsies and endoscopic examinations: the cutting head of the biopsy capsule did not open by suction; in some patients a decreased motility prolonged the transport time of the capsule through the stomach and duodenum, and the patients wished to stop the examination. These problems were solved using a higher suction pressure and by oral application of metoclopramid to increase gastrointestinal motility.

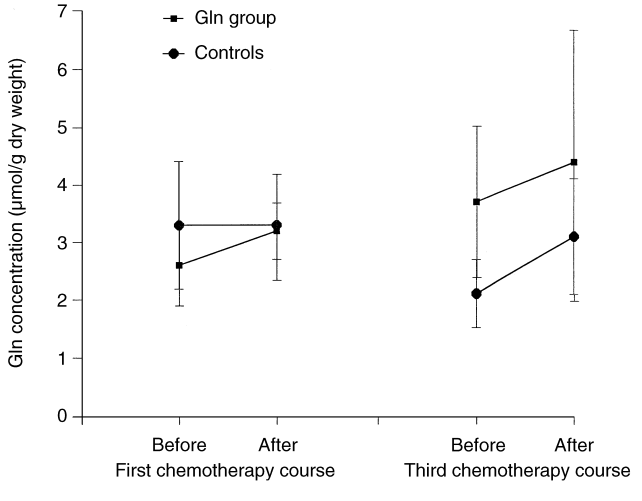

Figure 1. Glutamine (Gln) concentration in the duodenal mucosa. First chemotherapy course: Gln group $n = 8$, controls $n = 6$; third chemotherapy course: Gln group $n = 5$, controls $n = 7$. In the Gln group, the Gln concentration of the duodenal mucosa increased during the study course.

Before the first and third chemotherapy courses no significant differences were seen in the ratio of villus height to crypt depth (v:c ratio) between both groups. During chemotherapy the v:c ratio decreased slightly in controls, but increased in the Gln group (Table 3). After the first and third chemotherapy courses the v:c ratio was significantly higher in the Gln group than in the controls (first chemotherapy course $P = 0.01$; third chemotherapy course $P = 0.03$; Figure 2). The incidence of mucositis and ulcerations of the gastric (Figure 3) and duodenal mucosa (Figure 4) was lower in the Gln group, than in controls *P* values. One patient in the Gln group and 5 patients in the control group suffered

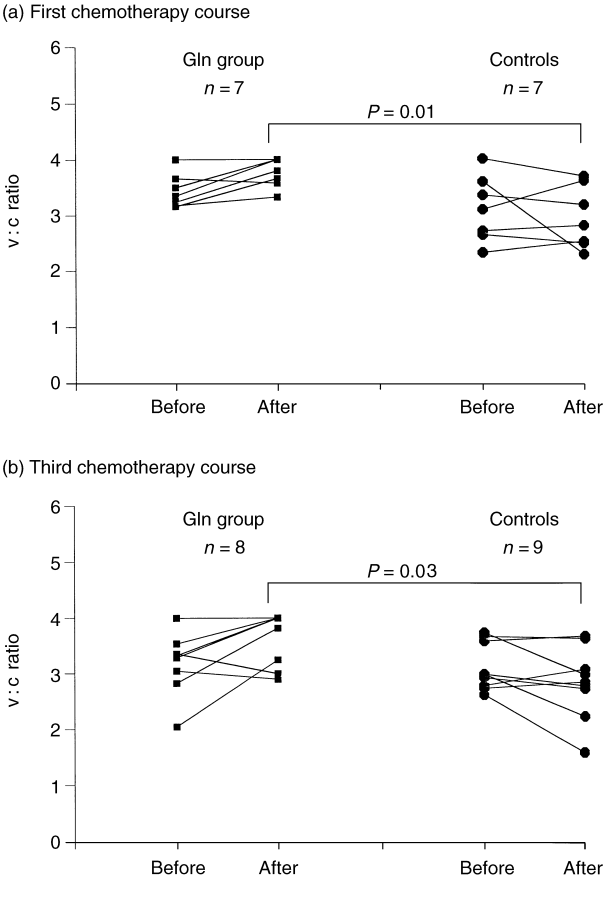

Figure 2. Ratio of villus height to crypt (v:c) depth before and after chemotherapy. (a) First chemotherapy course: Gln group $n = 7$, controls $n = 7$. $P = 0.01$, Gln group after chemotherapy versus controls after chemotherapy; (b) third chemotherapy course: Gln group $n = 8$, controls $n = 9$. $P = 0.03$, Gln group after chemotherapy versus controls after chemotherapy. Before treatment there were no significant differences between the groups for the v:c ratio, but after the first and third chemotherapy courses, the ratio was significantly higher in the Gln group ($P < 0.05$).

from clinical side-effects with WHO grade 3. However, in total no significant differences between the groups in the incidence and severity of clinical side-effects according to the WHO grading were observed (Figure 5).

DISCUSSION

Chemotherapy is compromised by side-effects such as gastrointestinal mucositis followed or accompanied by nausea, vomiting, lack of appetite and diarrhoea. The clinical symptoms correlate with chemotherapy-induced damage of the rapidly proliferating cells of the gastrointestinal tract [11]. Under these conditions, nutrient intake and absorption are reduced and patients often suffer from malnutrition. Animal and human studies have shown that Gln depletion results in structural and functional alterations of the gastrointestinal mucosa, which can be prevented by Gln supplementation [3–5, 12–15]. Furthermore, in animals Gln-enriched enteral nutrition protects the intestine against chemotherapy-induced injuries of the mucosal structure [7].

Until now the appropriate Gln dosage to prevent chemotherapy-induced mucosal side-effects was unknown. Results from previous studies have shown that parenteral supplementation with 0.23 g Gln/kg body weight (approximately 13 g Gln/day) preserved mucosal structure in patients with inflammatory bowel disease and neoplastic disease [3]. An i.v. dose of 12 g Gln/day has been shown to prevent intestinal atrophy in intensive care unit patients [4]. The daily administration of 12–14 g Gln has been recommended to abolish trauma-induced muscle Gln depletion and improve nitrogen balance [16]. Considering chemotherapy-induced side-effects, such as vomiting and diarrhoea, the parenteral route for Gln supplementation was chosen in this study. This has the advantage of standardisation and allowed the application of Gln at a level of the estimated requirement in illness [3, 4, 16].

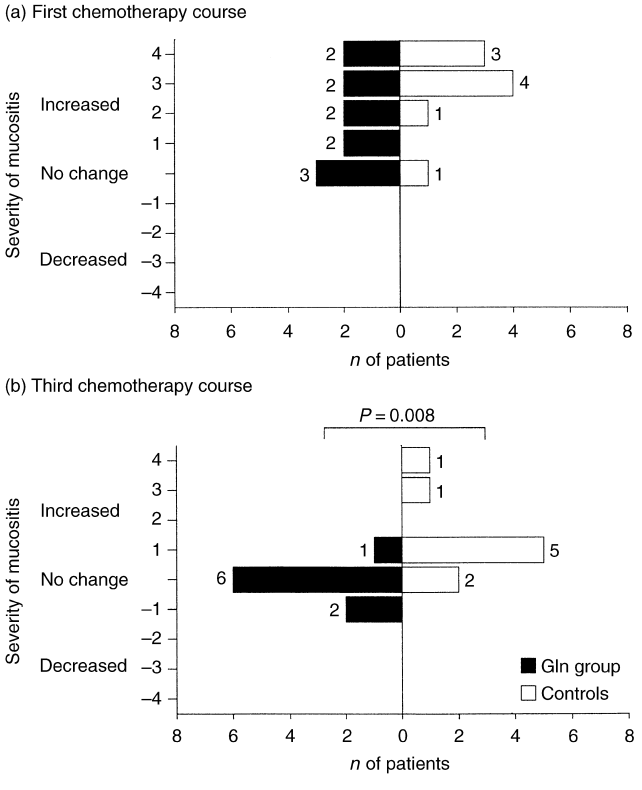

Figure 3. Comparison of gastroscopic findings before and after chemotherapy. (a) First chemotherapy course: Gln group $n = 11$, controls $n = 9$. During chemotherapy the incidence of gastric ulcerations and severe mucositis was lower in the Gln group; (b) third chemotherapy course: Gln group $n = 9$, controls $n = 9$. In the Gln group the gastroscopic findings remained unchanged during therapy, whereas in the controls there was a higher incidence of mucosal injury ($P = 0.008$, Gln group versus controls).

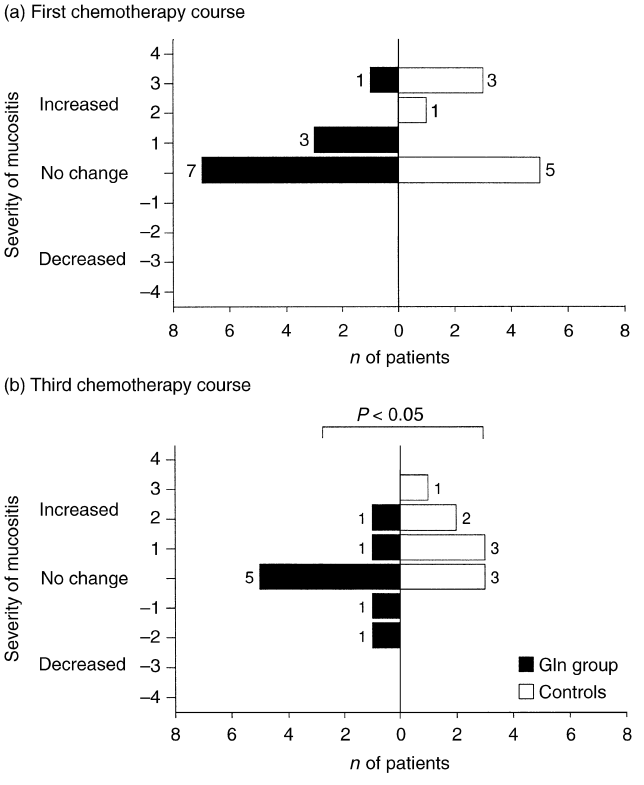

Figure 4. Comparison of duodenoscopic findings before and after chemotherapy. (a) First chemotherapy course: Gln group $n = 11$, controls $n = 9$; (b) third chemotherapy course: Gln group $n = 9$, controls $n = 9$. During the third course of chemotherapy, a lower incidence of mucosal injury of the duodenal bulb was observed in the Gln group than in the controls ($P < 0.05$, Gln group versus controls).

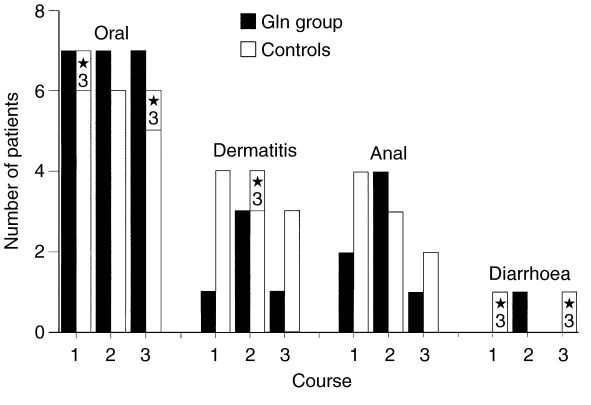

Figure 5. Clinical side-effects World Health Organisation—grades 2 and 3. No significant differences were seen between groups in the incidence and severity of clinical side-effects. A tendency towards a reduced incidence of dermatitis and anal mucositis was seen in the Gln group after chemotherapy. *n of patients with grade 3 toxicity.

In the present study, Gln was given as a supplement to oral nutrition. Therefore, oral food intake was assessed to differentiate Gln effects from other nutritional effects. The method used requires that all food is weighed prior to eating and leftovers are also weighed and subtracted. To differentiate among similar foodstuffs, all food was described in detail. These surveys have the advantage of accuracy and produce data that can be expressed in quantitative terms and converted into nutrients. This method is a reproducible and valid method to measure nutrient intake and serve as the basis of clinical research [17]. The nutrient intake in both groups differed only in the quantity of Gln supplementation, so that differences in the results can be correlated to Gln effects.

At the beginning of the study and during the study, the nutritional status of both groups was in the normal range. This is important because a positive effect of Gln supplementation so far has only been proven in malnourished patients [18]. In a previous study [19], the Gln-supplemented group of cancer patients given intensive chemotherapy gained significantly more weight per treatment cycle than the unsupplemented group. This could not be substantiated in the present study.

Plasma Gln concentrations were similar in both groups and comparable with healthy individuals [20] and non-depleted patients [5]. In contrast to other investigations [18, 20], which examined Gln concentration during infusion, the present study did not show an increased Gln concentration on the morning following the last day of supplementation. Since Gln uptake by the gastrointestinal tract and other Gln metabolising cells is known to be concentration dependent, these findings could be interpreted as a normal physiological process [21]. The present data confirmed that plasma Gln concentrations did not change after Gln dipeptide infusion, whereas the Gln content of the duodenal mucosa increased after Gln supplementation. Corresponding to the increased duodenal Gln concentration, a significant improvement was seen in the morphological appearance of the mucosa determined by the v:c ratio. Villus height and crypt depth are relevant parameters with which to judge the severity of gastrointestinal cell damage.

Animal studies have demonstrated that Gln-enriched elemental diets decreased methotrexate-induced intestinal injuries [6, 7] and that oral Gln accelerated repair of the small intestine following whole abdominal radiation [22]. In the present study, the endoscopic findings showed a significant prevention of chemotherapy-associated duodenal injuries in humans. Previous studies investigated the protective effects of oral Gln on gastric mucosa. In rats Gln inhibited acetylsalicylic acid-induced gastric ulcers [23]. Shive and colleagues [24] reported on the treatment of peptic ulcers in humans with oral Gln supplementation. Thus, it is not surprising that the present results showed that Gln supplementation significantly reduced chemotherapy-induced gastric mucositis or ulcerations.

Van Zaanen and co-workers [19] could not find significant differences between the Gln-supplemented and unsupplemented patients in the incidence of chemotherapy-induced side-effects and toxicity scores. Their study was also based on a small number of patients and median level of mucositis and diarrhoea corresponded to the WHO grade 0–0.3. As such, the present study was also unable to demonstrate significant differences between groups in the severity and incidence of clinical side-effects. All of the patients suffered from stomatitis. These data are difficult to interpret. To the authors' knowledge, no data are available on the activity of the glutaminase enzyme in the oral mucosa of humans. It seems that, in contrast to the intestinal mucosa, Gln does not influence the function and structure of these cells. However, some reduction of anal mucositis was observed in the Gln group. Analogous to the oral mucosa, no data are available about the glutaminase activity in the anal mucosa; however, it has been demonstrated that Gln has trophic effects in the proximal and rectosigmoid colon [25]. Therefore, the lower incidence of anal mucositis in the supplemented group may be related to Gln. The lower incidence of dermatitis in the Gln group may also be related to the supplementation. An explanation has been provided by Rouse and colleagues [26], who demonstrated a mechanism by which Gln supplementation decreases toxicity to normal tissues and increases damage to tumour cells in rats treated with methotrexate: there is an upregulation of glutathione metabolism in normal tissues and a downregulation in tumour cells.

The present findings showed that a similar protective effect of Gln supplementation to the gastrointestinal mucosa seen in animals can also be expected in patients receiving 5-FU/CF chemotherapy. The endoscopic and histomorphometric results are encouraging and should be confirmed in larger clinical trials. However, the patients were not malnourished and the therapy generally showed very few side-effects. Therefore, in malnourished patients receiving highly toxic chemotherapy or a combination of radiotherapy and chemotherapy, parenteral supplementation with Gln could significantly reduce the clinical side-effects of an aggressive treatment schedule.

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