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Clinical trial: prophylactic intravenous alanyl-glutamine reduces the severity of gastrointestinal toxicity induced by chemotherapy – a randomized crossover study

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SUMMARY

Background
Glutamine has been shown in numerous studies to reduce intestinal permeability which can be increased by chemotherapy. However, there have been few reports that conduct on its clinical effect on gastrointestinal toxicity.

Aim
To examine whether prophylactic intravenous alanyl-glutamine dipeptide can ameliorate clinical manifestations of gastrointestinal toxicity induced by chemotherapy.

Methods
Forty-four patients with gastric or colorectal cancer developing WHO side-effect grading system of grade 2 or higher were randomized to either control group (n = 22) or Gln group (n = 22) during next cycle of chemotherapy. Patients were crossed over to the alternate treatment during chemotherapy cycle 2. In the control group, the patients received the same chemotherapy regimens as screening cycle and in the Gln group, the patients received chemotherapy and alanyl-glutamine. Prophylactic intravenous 20 g of alanyl-glutamine dipeptide was given for 5 days.

Results
Compared with the control group, the plasma glutamine level in the Gln group was significantly higher and the plasma endotoxin level was significantly lower. The scores of nausea/vomiting and diarrhoea decreased significantly.

Conclusion
Prophylactic intravenous alanyl-glutamine is effective in preventing intestinal permeability disruption induced by chemotherapy and clinical manifestations of gastrointestinal toxicity.

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INTRODUCTION

Treatment of malignant tumours with cytotoxic chemotherapy is becoming increasingly more and more effective, but it is associated with side effects.[1–3] Among the clinically important acute side effects is the disruption in the function and integrity of the intestine.[4] Cytotoxic therapy-induced intestinal epithelial damage is associated with a variety of clinical complications, including oropharyngeal mucositis, abdominal pain, diarrhoea, electrolyte imbalance, bleeding risk, neutropenic enterocolitis and invasive infection resulting from the translocation of endogenous microorganisms colonizing gastrointestinal surfaces.[5–7]

In contrast to the relative ease of observing oral changes as a consequence of chemotherapy, defining the impact on gut function is difficult because of its inaccessibility and lack of appropriate non-invasive techniques to assess the severity of damage. In animal studies, viable indigenous bacteria from the gut lumen to extraintestinal organs had been confined by tissue histology, culture techniques and radioactivity. However, the methods used in most animal studies could not provide direct evidence that translocating bacteria are really derived from intestinal microbial flora. We had confirmed that methotrexate can induce *Escherichia coli* TG1 labelled with green fluorescent protein (GFP) translocation from the gut to the mesenteric lymph nodes, spleen, liver and kidney in a rat model of chemotherapy.[8] In clinical practice, impairment of gut function and small intestinal barrier integrity have been measured using a test for small intestinal permeability; others studies, as well as ours, had demonstrated that chemotherapy can induce increase in intestinal permeability.[7–18]

Glutamine is the most abundant free amino acid in the body. It was shown to be the major respiratory fuel for the intestinal tract.[19] There have been numerous studies on the effects of glutamine on intestinal mucosal damage, which is induced by chemotherapy.[7, 10–12] We had demonstrated that prophylactic glutamine could decrease the neoadjuvant chemotherapy-induced increase in intestinal permeability. However, most clinical trials including our previous study did not observe improvement of clinical manifestations of gastrointestinal toxicity. In this study, we investigated whether prophylactic intravenous alanyl-glutamine can ameliorate severity of gastrointestinal toxicity induced by chemotherapy in patients with gastrointestinal cancer.

MATERIALS AND METHODS

Patient eligibility

Patients enrolled were required to meet the following eligibility criteria: (i) diagnosis with gastric or colorectal cancer; (ii) WHO-developed side-effect grading system of grade 2 or higher in previous screening chemotherapy cycle; (iii) aged between 40 and 69 years.

The exclusion criteria were: (i) glutamine allergy; (ii) abdominal-pelvic radiotherapy in their medical history; (iii) renal and/or function insufficiency; (iv) administration of antibiotic therapy for specific indication of fever; (v) use of analgesics or/and anti diarrheic. This study was approved by the Ethics Committee of Nanjing University and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Study design

The study used a randomized, double-blind, crossover design to evaluate glutamine in gastric or colorectal cancer patients with grade 2 or higher of World Health Organization side-effect grading system. Forty-four patients (twenty-five men and seventeen women) with a mean age of 56.2 years (range 46–68 years) were enrolled in this study. All patients receiving chemotherapy scored grade 2 or higher during a chemotherapy screening cycle were randomized to either control group (n = 22) or Gln group (n = 22) during next cycle of chemotherapy (chemotherapy cycle 1). Patients were crossed over to the alternate treatment during chemotherapy cycle 2 (Figure 1). In the control group, the patients received the same chemotherapy regimens as the last cycle and in the Gln group, the patients received chemotherapy and alanyl-glutamine. Prophylactic intravenous 20 g (about 0.3 g/kg/day) of alanyl-glutamine dipeptide (Dipeptiven; Fresenius-Kabi, Bad Homburg, Germany) was given on day 1 of chemotherapy and continued for 5 days.

Chemotherapy protocol

The patients received chemotherapy including FAM (5-FU 600 mg/m^2, IV, d1–d5; doxorubicin 30 mg/m^2, IV, d1; mitomycin 10 mg/m^2, IV, d1) regimens (n = 26) in gastric cancer patients and FOLFOX-4 (oxaliplatin 85 mg/m^2, d1; folinic acid 200 mg/m^2, d1, 2; 5-FU 400 mg/m^2 bolus + 600 mg/m^2 infusion over 22 h, d1, 2) regimens in colorectal cancer patients

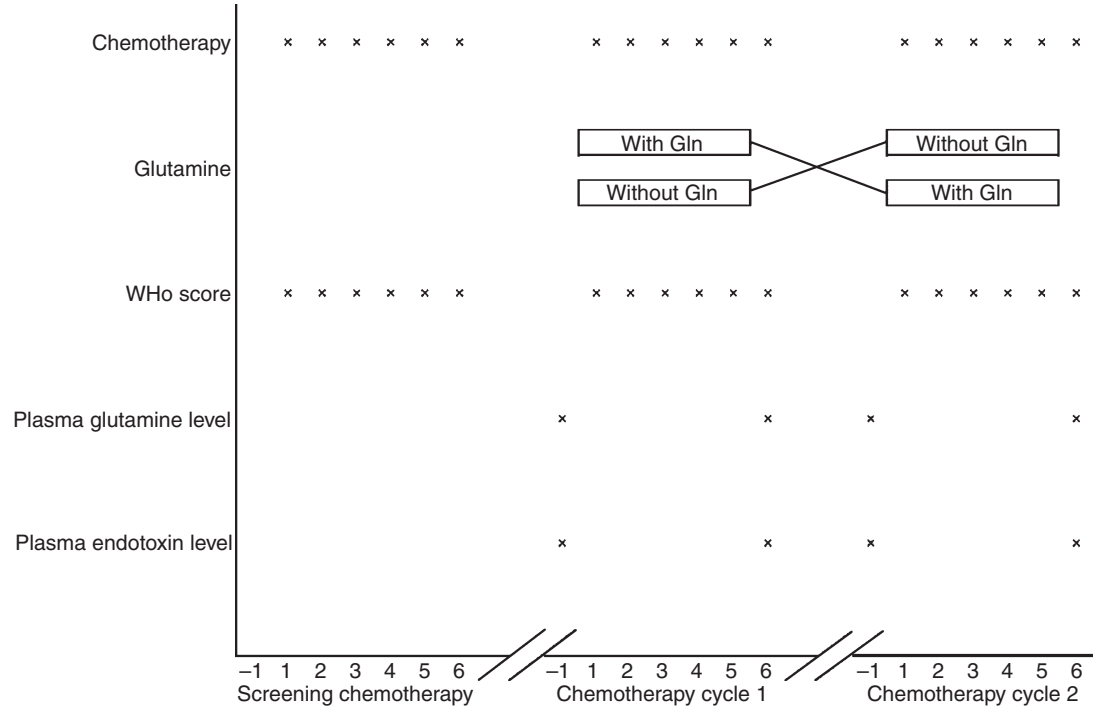

Figure 1. Trial protocol.

(n = 18). A new course of treatment could begin if the absolute neutrophil count was or more than 1500/mm^2, the platelet count was or more than 100 000/mm^3. If, after a 1-week delay, toxicities were or less than grade 1, treatment resumed. If the toxicity did not resolve in 1 week, a second one-week delay was allowed. Recombinant human granulocyte colony-stimulating factor was administered for patients with leucopenia, neutropenia and thrombocytopenia. No patient had treatment interrupted lasting more than 3 weeks. The patient characteristics are shown in Table 1.

Table 1. Baseline characteristics of patient

	Without Gln (n = 22)	With Gln (n = 22)
Gender (male:female)	11:11	13:9
Age (years)	56.5 ± 6.2 (46–68)	56.1 ± 5.9 (48–68)
Weight (kg)	74.3 ± 8.9 (48–86)	72.4 ± 9.4 (52–84)
Diagnosis		
Gastric cancer	14	16
Colorectal cancer	8	6
Scales of gastrointestinal toxicity during of screening cycle		
Nausea/vomiting	2.59 ± 0.13	2.61 ± 0.15
Diarrhoea	2.76 ± 0.19	2.69 ± 0.20

Endpoints

Patients were asked by Dr Ping to grade and record on a daily basis, symptoms of nausea, vomiting, diarrhoea from the first chemotherapy day until the sixth chemotherapy day. Grading of symptoms was based on WHO side-effect grading system (Table 2). Intestinal permeability (lactulose–mannitol test) was not measured because of the impact of diarrhoea by lactulose and mannitol.

Table 2. Gastrointestinal toxicity

	Without Gln (n = 22)	With Gln (n = 22)
Nausea/vomiting		
Screening chemotherapy	2.59 ± 0.13	2.61 ± 0.15
Study chemotherapy	2.63 ± 0.21	1.18 ± 0.31*,†
Diarrhoea		
Screening chemotherapy	2.76 ± 0.19	2.69 ± 0.20
Study chemotherapy	2.82 ± 0.34	1.31 ± 0.25*,†

* $P < 0.05$ compared with control group.
† $P < 0.05$ compared with screening chemotherapy.

Blood sampling and handling

All blood samples were taken from peripheral veins. Plasma glutamine and endotoxin levels were measured on the first and the sixth day of each course. Because of the instability of glutamine in the blood samples, the samples were centrifuged immediately and the supernatant was frozen prior to analysis.

Plasma glutamine concentration

Plasma glutamine was measured using the method of high-performance liquid chromatography as described previously.[20]

Plasma endotoxin concentration

Plasma endotoxin was measured as described previously.[21]

Additional measurements

Routine haematology and biochemistry tests were performed prechemotherapy and on the third and sixth chemotherapy day.

Statistical analysis

Measurements were averaged and are expressed as mean ± standard deviation. Data were entered into a computerized database (SPSS statistical software, SPSS Inc., Chicago, IL and MINITAB statistical software, Minitab Inc., State College, PA). The paired *t*-test was used for parametric data and Mann–Whitney *U*-test for nonparametric variables to compare data from the two phases of the study. Statistical significance was accepted at the $P < 0.05$ levels.

RESULTS

The clinical characteristics of patients in both groups were similar at entry (Table 1).

Plasma glutamine concentration

Figure 2 shows the plasma glutamine levels. In the control group, the plasma glutamine level was decreased after chemotherapy when compared with prechemotherapy, although the difference was not significant ($P > 0.05$). The glutamine level in the Gln group was significantly higher than in the control group ($P < 0.05$).

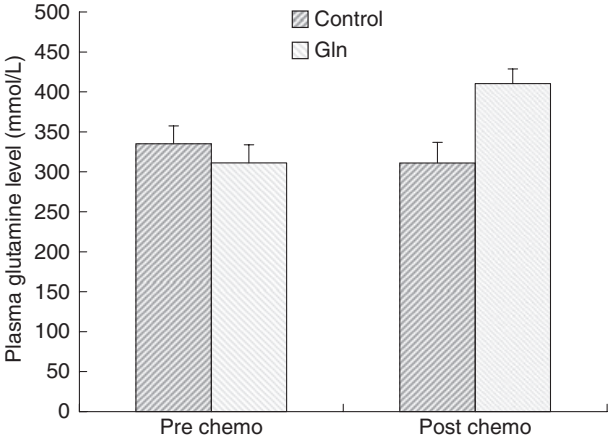

Figure 2. Plasma glutamine concentration. The glutamine level was higher in Gln group than in control group.

Plasma endotoxin concentration

Figure 3 shows the plasma endotoxin levels. In the control group, the plasma endotoxin level increased following chemotherapy ($P < 0.05$). In the Gln group, the plasma endotoxin level after chemotherapy also

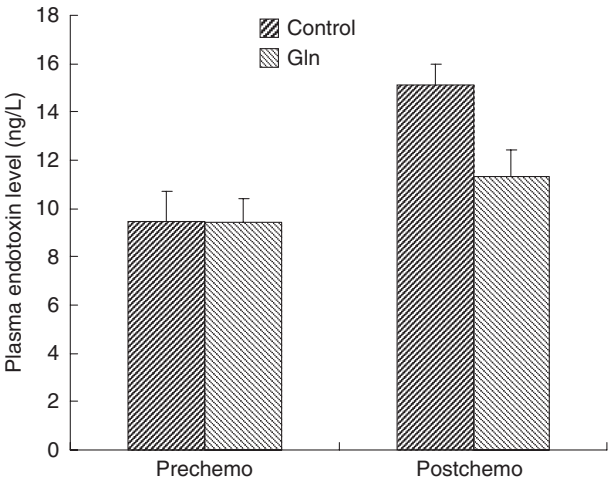

Figure 3. Plasma endotoxin levels. In the control group, the plasma endotoxin level was higher than that of prechemotherapy ($P < 0.05$). In the Gln group, there was no significant difference ($P > 0.05$).

increased compared with prechemotherapy; however, there was no significant difference ($P > 0.05$).

Haematology and biochemistry observations

There were no significant difference in leucopenia, neutropenia and thrombocytopenia and dose of recombinant human granulocyte colony-stimulating factor in the two groups. In Biochemistry tests, there were no significant differences in the two groups (data not shown).

Gastrointestinal toxicity

Table 2 shows the gastrointestinal toxicity. The scores of nausea/vomiting and diarrhoea were significantly lower in the Gln group than in the control group. And in the Gln group, the scores of nausea/vomiting and diarrhoea were significantly lower than those of screening chemotherapy.

DISCUSSION

Millions of patients each year accept intensive chemotherapy, which is often complicated by damage to the mouth and gastrointestinal mucosa.[18] Any part of the gastrointestinal tract from the oral cavity to the anus may be affected. This indiscriminate cytotoxicity results in a cascade of side effects where cells, particularly those with a rapid cell-turnover rate, are highly susceptible to damage. Consequently, the gastrointestinal barrier function capacity diminished.[17] The physiological intestinal barrier is formed primarily by the mechanical cell barrier and intercellular junctions, the immunological barrier, normal microbial flora and the liver–intestine axis.[22] Alterations in all of these components of the intestinal barrier have been reported to be responsible for bacterial and toxin translocation.[23]

The intestinal barrier dysfunction has been demonstrated to be associated with an increased incidence of bacteria and toxin translocation from the intestinal lumen to the systemic circulation, causing complications of infection in critically ill patients[24–26] and clinical outcomes during remission–induction therapy in acute myeloid leukaemia.[27] So, how to maintain the intestinal barrier during chemotherapy was a challenge for physicians.

The intestinal permeability increase caused by chemotherapy, shock and burn had been demonstrated in laboratory animals on the basis of monitoring bacterial migration using tissue histology, microbial culture of internal organs.[28–30] However, definitive evidence of bacterial translocation is lacking. The tissue histology, microbial culture did not provide direct evidence that translocating bacteria are really derived from intestinal microbial flora. Our previous study provided direct evidence of bacterial translocation and intestinal barrier dysfunction induced by methotrexate. Intestinal barrier dysfunction were induced on Sprague–Dawley rats by 3-day's treatment of methotrexate (3.5 mg/kg). The rats were gavaged *E. coli* TG1 labelled with GFP. Intestinal permeability was measured by the urinary excretion rate of lactulose and mannitol. Two days later, *E. coli* labelled with GFP from mesenteric lymph nodes, liver, spleen and kidney were isolated. Intestinal permeability (lactulose–mannitol test) was increased by chemotherapy.[8] In this study, lactulose–mannitol test was not used for measurement of intestinal permeability, oral lactulose and mannitol can lead to diarrhoea, which has impact on observation of WHO side-effect grading system. Hence, we measured the plasma endotoxin levels to assess the intestinal barrier function. In the control group, the plasma endotoxin level was increased after chemotherapy when compared with that before chemotherapy; there was no significant difference between prechemotherapy and postchemotherapy in the Gln group ($P > 0.05$).

While mucosal damage in the oral cavity can be easily assessed by direct inspection, involvement of other regions of the gastrointestinal tract may be evaluated solely by endoscopy, which is less easily performed in a patient coping with the side effects of anti-cancer therapy. Functional measures of the integrity of the intestinal epithelial barrier using orally administered saccharide or radiolabelled probes have been applied to the study of gastrointestinal mucositis among patients receiving chemotherapy and stem cell transplant recipients.[31–34]

Animal and clinical studies have been reported using probiotics,[4] granulocyte colony stimulating factor,[8] glutamine,[7, 11, 12, 35] enteral nutrition,[36] transforming growth factor, glutamine and short chain fatty acid[37] and IgA–IgG.[33] Protective effect of glutamine that prevents and/or minimizes an acute increase in intestinal permeability-induced by chemotherapy had been investigated in several trials[7, 9–12, 35, 36] and nonsteroid anti-inflammatory drug.[38] However, most studies had confirmed that glutamine ameliorates

intestinal permeability evaluated by the surrogates index, such as lactulose–mannitol test. Previous studies including our study did not show that the glutamine improved clinical manifestations of gastrointestinal toxicity induced by chemotherapy in patients who had no history of gastrointestinal toxicity before chemotherapy. In this study, the patients with gastrointestinal toxicity of grade 2 or higher during last chemotherapy were enrolled in the investigation, the objective being to study the severe adverse effects caused by chemotherapy. Our results indicated that alanyl-glutamine dipeptide decreased clinical manifestations of gastrointestinal toxicity in patients who had gastrointestinal toxicity of grade 2 or higher scored according to the to WHO side-effect grading system.

The pathogenesis of mucositis induced by chemotherapy is complex. It was believed that direct damage by chemotherapy to the basal epithelial cell layer led to loss of the renewal capacity of the epithelium. It is, however, becoming clear that mucositis is linked to other nonmucosal toxicities of anticancer therapy, such as fatigue, malnutrition and nausea.[2] Glutamine is the most abundant free amino acid in the human body and is essential for the growth of normal and rapid proliferation cells. The mechanism by which glutamine ameliorate mucositis induced by chemotherapy remains uncertain. It was considered that glutamine is a preferred fuel for the enterocyte and can increase intestinal epithelial cell proliferation. Currently, the mechanisms of glutamine on the improvement of gastrointestinal toxicity has been investigated. Sornsuvit *et al.*[2] demonstrated the parenteral Gln supplementation enhances neutrophil phagocytic function and prevention of chemotherapy-induced side effects in acute myeloid leucaemia patients. It was demonstrated that glutamine accelerates the mucosal recovery increasing mucosal tissue glutathione stores and speeding re-epithelization on the 14th day, but did not prevent oral mucositis on the 10th day.[10] Kaufmann *et al.*[39] confirmed that that oral glutamine suppresses DMBA-induced mammary carcinogenesis by upregulation of glutathione production and augmentation of NK cell activity. The intestinal epithelium serves as an important intestinal barrier, which was damaged by chemotherapy. Kessel *et al.*[40] showed that Gln down regulates Toll-like receptor-4, myeloid differentiation primary response gene 8 expression and concomitant decrease in intestinal mucosal injury caused by LPS endotoxemia.

In conclusion, prophylactic intravenous alanyl-glutamine is effective for preventing intestinal permeability disruption induced by chemotherapy and clinical manifestations in gastrointestinal toxicity in patients who had WHO side-effect grading system of grade 2 or higher during a chemotherapy screening cycle.

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