PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(39):6197-6201
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Glutamine-supplemented total parenteral nutrition attenuates plasma interleukin-6 in surgical patients with lower disease severity

Ming-Tsan Lin, Sung-Pao Kung, Sung-Ling Yeh, Koung-Yi Liaw, Ming-Yang Wang, Ming-Liang Kuo, Po-Houng Lee, Wei-Jao Chen

Ming-Tsan Lin, Department of Surgery and Primary Care Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, China
Sung-Pao Kung, Department of Surgery, School of Medicine, National Yang-Ming University, Taipei, Taiwan, China
Sung-Ling Yeh, Institute of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan, China
Koung-Yi Liaw, Ming-Yang Wang, Po-Houng Lee, Wei-Jao Chen, Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, China
Ming-Liang Kuo, Laboratory of Molecular and Cellular Toxicology, Institute of Toxicology, National Taiwan University College of Medicine, Taipei, Taiwan, China
Supported by a Research Grant from the National Science Council, Taipei, Taiwan, No. NSC91-2314-B002-245
Co-correspondents: Sung-Pao Kung
Correspondence to: Wei-Jao Chen, MD, PhD, Department of Surgery, National Taiwan University Hospital, 7 Chung-Shan S. Road, Taipei, Taiwan, China. chenwj@ha.mc.ntu.edu.tw
Telephone: +886-2-23123456-2122 Fax: +886-2-23412969
Received: 2005-02-15 Accepted: 2005-06-09

Abstract

AIM: To evaluate whether the effect of Gln dipeptide-enriched total parenteral nutrition (TPN) on postoperative cytokine alteration depended on the disease severity of surgical patients.

METHODS: Forty-eight patients with major abdominal surgery were allocated to two groups to receive isonitrogenous (0.228 g nitrogen/kg per d) and isocaloric (30 kcal/kg per d) TPN for 6 d. Control group (Conv) using conventional TPN solution received 1.5 g amino acids/kg per day, whereas the test group received 0.972 g amino acids/kg per day and 0.417 g L-alanyl-L-glutamine (Ala-Gln)/kg per day. Blood samples were collected on d 1 and d 6 postoperatively for plasma interleukin (IL)-2, IL-6, IL-8, and interferon (IFN)-γ analysis.

RESULTS: Plasma IL-2 and IFN-γ were not detectable. IL-6 concentrations were significantly lower on the 6th postoperative day in the Ala-Gln group than those in the Conv group in patients with APACHE II≤6, whereas no difference was noted in patients with APACHE II>6. There was no difference in IL-8 levels between the two groups. No difference in cumulative nitrogen balance was observed on d 2-5 after the operation between the two groups (Ala-Gln -3.2±1.6 g *vs* Conv -6.5±2.7 g). A significant inverse correlation was noted between plasma IL-6 levels and cumulative nitrogen balance postoperatively in the Ala-Gln group, whereas no such correlation was observed in the Conv group.

CONCLUSION: TPN supplemented with Gln dipeptide had no effect on plasma IL-8 levels after surgery. However, Gln supplementation had a beneficial effect on decreasing systemic IL-6 production after surgery in patients with low admission illness severity, and lower plasma IL-6 may improve nitrogen balance in patients with abdominal surgery when Gln was administered.

Key words: Glutamine; Total parenteral nutrition; Interleukin-6; Abdominal surgery

Lin MT, Kung SP, Yeh SL, Liaw KY, Wang MY, Kuo ML, Lee PH, Chen WJ. Glutamine-supplemented total parenteral nutrition attenuates plasma interleukin-6 in surgical patients with lower disease severity. *World J Gastroenterol* 2005; 11(39): 6197-6201
http://www.wjgnet.com/1007-9327/11/6197.asp

INTRODUCTION

Glutamine (Gln) is the most abundant amino acid in plasma and in the intracellular free amino acid pool[1]. It is essential for a wide variety of physiologic process, in particular, the growth and function of immune cells including lymphocytes and macrophages[2]. Gln has traditionally been thought as a nonessential amino acid; however, previous reports have shown that Gln depletion occurs in critically injured patients. The extent and duration of Gln depletion are proportional to the severity of illness[3,4]. Laboratory and clinical data suggest that Gln is essential during certain catabolic conditions, such as burns, major surgery, and infection[5-8]. Total parenteral nutrition (TPN) is widely used in the treatment of critically ill patients. Conventional TPN solution does not contain Gln, because Gln is relatively unstable in solution during heat sterilization and long-term storage. Exogenous Gln may be required to satisfy the body's Gln requirement under stressed conditions. Previous studies have shown that TPN supplemented with Gln improved nitrogen balance, enhanced immune response and shortened hospital stay in surgical patients[9-12].

Surgery is known to impair the immune response, and may consequently increase risk of postoperative infection and sepsis[13]. The decrease in the plasma Gln

level following major surgery may contribute to the state of immunosuppression[12,14]. Studies have shown that depressed Gln concentrations were associated with reduced proliferation of lymphocyte in healthy volunteers[14], and Gln depletion may be partly responsible for T cell suppression seen in severely stressed patients[15,16]. In addition to its role in lymphocyte function, Gln may have an indirect effect to mediate a reduction in proinflammatory cytokine release. Parry-Billings *et al*.[3], demonstrated that plasma Gln level was negatively correlated with the production of interleukin (IL)-6 under major surgery. De Beaux *et al*.[17], showed that Gln-supplemented TPN significantly reduced blood mononuclear cell IL-8 release in severe acute pancreatitis patients. A previous report by our laboratory have shown that the effect of Gln administration on improving nitrogen economy was only observed in patients with low, but not high Chronic Health Evaluation (APACHE II) scores[11]. This result indicates that the effect of Gln on attenuating catabolic response may depend on the characteristics and severity of the diseases. We hypothesized that the effect of synthetic Gln supplementation on proinflammatory cytokine release was also associated with the severity of diseases. In this study, we administered a Gln-dipeptide containing TPN to patients postoperatively to investigate the effect of synthetic Gln-dipeptide on cytokine production after surgery. Also, the Gln effect on the severity of stress and cytokine production was evaluated.

MATERIALS AND METHODS

Patients

This was a randomized, double-blind, parallel multicenter clinical trial carried out in National Taiwan University Hospital and Veterans General Hospital, Taipei, Taiwan. This study was performed from August 1999 to May 2000, and was approved by the ethical committee of the two centers. Patients with major metabolic, circulatory, and renal diseases were excluded, and no emergency cases were included. Only major gastrointestinal surgery patients who needed TPN for nutritional support were enrolled. APACHE II score and Therapeutic Intervention Scoring system (TISS) were evaluated after admission[18]. Patients with APACHE II between 2-10 and TISS>10 were included. The informed consent was obtained from each patient before the experiment was performed. A total of 48 patients (28 males and 20 females, age range 40-82 years, mean 66 years) were included. These patients were randomly allocated to either a test group or a control group. The separation of patients into low and high APACHE II groups was post hoc. Since patients with APACHE II score between 2 and 10 were enrolled, we chose the midpoint 6 as the cut-off point to differentiate low- and high-disease severity in this study. Demographic for all patients is summarized in Table 1.

Feeding regimen

The two groups were isonitrogenous and isocaloric. Nitrogen intake was 0.228 g/kg body weight per ds. Caloric intake was 30 kcal/kg per ds. The nonprotein calories were given as dextrose and fat in a ratio of 70:30. Patients of the control group (Conv group, n = 23) were administered a commercially available amino acid solution (Moriamin-SN 10%, Chinese Pharmaceuticals, Taipei, Taiwan). The Conv group received 1.5 g amino acid/kg per day. Patients of the test group (L-alanyl-L-glutamine (Ala-Gln) group, n = 25) received 0.972 g amino acid/kg per day supplemented with 0.417 g/kg per ds Ala-Gln, which provide 0.28 g Gln/kg per day (Dipeptiven, Fresenius Kabi, Bad Homburg, Germany). The Gln containing solutions were prepared by the clinical pharmacist under aseptic condition and adjusted to the weight of each individual patient. The amino acids and dextrose mixture with electrolytes, vitamins, and trace elements were administered through a central venous catheter. Fat emulsion (Lipovenos 20%, Fresenius AG, Germany) was given through a separate canal in the central venous line. Half strength was administered on the 1st d, and full strength was given thereafter for the remaining days. During the experimental period, no enteral nutrition was administered. Neither the patients nor the investigator knew that the applied TPN regimens were with or without Ala-Gln.

Measurements of the plasma parameters and cumulative nitrogen balance

Routine clinical chemistry was measured before and after the surgery. The blood samples were also obtained on postoperative d 1 and on d 6 after TPN administration for analysis of plasma cytokine concentrations. Cytokines including IL-2, IL-6, IL-8, and interferon (IFN)-γ were measured using a commercially available ELISA in microtiter

Table 1 Demographic data of the patients

	Ala-Gln group			Conv group		
	All patients	APACHE ≤6	APACHE >6	All patients	APACHE ≤6	APACHE >6
Age (yr)	66.7±9.0	61.7±11.2	70.1±6.0	67.6±8.4	64.5±7.6	70.6±8.7
Weight (kg)	56.9±9.1	57.7±8.6	56.3±10.1	57.6±10.3	55.3±8.6	59.8±12.0
Height (cm)	157.8±6.4	160.4±6.7	156.0±6.1	160.1±8.3	158.8±8.0	161.3±9.0
Male/female	14/11	4/6	10/5	14/9	6/5	8/4
Diagnosis:						
Gastric Ca	18	6	12	14	6	8
Pancreas Ca	4	3	1	4	2	2
Colon Ca	1	0	1	3	2	1
Hepatoma	1	1	0	1	1	0
Duodenal Ca	0	0	0	1	0	1
Rectal Ca	1	0	1	0	0	0

plates (BioSource International, Camarillo, CA, USA). Antibody specific for human IL-2, IL-6, IL-8, and IFN-γ were coated onto the wells of microtiter stripes provided. Urine and drainage was collected throughout the study, and cumulative nitrogen balance was calculated from postoperative d 2-5 as previously described[11].

Statistics

The data are expressed as mean±SD, repeated measure analysis of variance were used to compare the treatment differences at each day, and *t*-test was used for the difference between the two groups. Pearson's correlation coefficients were used to calculate the linear relationship between plasma IL-6 and cumulative nitrogen balance of the patients. A *P* value <0.05 was considered significant.

RESULTS

Most of the patients had laboratory values within normal range at baseline and on d 6 after the operation despite the patients being administered with Conv or Ala-Gln solution (data not shown). No adverse reactions were found after the Gln-dipeptide containing solution was administered.

Plasma IL-2 and IFN-γ levels were not detectable. Plasma IL-6 concentrations were significantly lower after TPN administration for 6 d, regardless whether the patients were in Conv group or Ala-Gln group. In patients with APACHE II≤6, plasma IL-6 concentrations on the postoperative d 6 in the Ala-Gln group were significantly lower than those in the Conv group. However, there were no differences in IL-6 concentrations between the two groups after infusing TPN for 6 d in patients with APACHE II>6 (Table 2). No significant difference in plasma IL-8 concentrations were observed between the postoperative d 6 and d 1, regardless whether the patients were infused with Ala-Gln or Conv solution. Also, there were no differences in IL-8 levels between the two groups on d 6 and 1 postoperatively (Table 3).

There was no difference in cumulative nitrogen balance on days 2-5 after the operation between the two groups (Ala-Gln -3.2±1.6 g *vs* Conv -6.5±2.7 g). A significant inverse correlation was observed between plasma IL-6 levels on d 6 and cumulative nitrogen balance postoperatively in the Ala-Gln group ($P = 0.027$), whereas no such correlation was noted in the Conv group ($P = 0.919$) (Table 4).

Table 2 Plasma IL-6 concentrations on d 1 postoperatively and on d 6 after TPN administration

	Conv (pg/mL)	Ala-Gln (pg/mL)
All patients	(23)	(25)
D 1[1]	38.8±31.5	52.5±48.7
D 6	12.4±12.1[a]	7.9±5.9[a]
APACHE II≤6	(11)	(10)
D 1	31.2±21.8	37.1±35.4
D 6	11.9±10.5[a]	3.2±1.9[a,b]
APACHE II>6	(12)	(15)
D 1	45.1±41.7	59.8±53.4
D 6	12.9±14.8[a]	16.1±17.2[a]

[1]D 1 represents the data obtained after TPN administration. Figures in parentheses indicate number of patients. [a]P<0.05 *vs* d 1 after Surgery. [b]$P = 0.029$ from Conv group D 6 in APACHE II ≤ 6.

Table 3 Plasma IL-8 concentrations on d 1 postoperatively and on d 6 after TPN administration

	Conv (pg/mL)	Ala-Gln (pg/mL)
All patients	(23)	(25)
D 1[1]	18.9±8.4	37.6±46.1
D 6	28.3±22.4	64.6±62.8
APACHE II≤6	(11)	(10)
D 1	19.4±9.9	15.6±9.0
D 6	36.9±45.9	24.5±8.9
APACHE II>6	(12)	(15)
D 1	18.5±7.3	48.4±52.3
D 6	31.0±30.4	81.3±67.9

[1]D 1 represents the data obtained before TPN administration. Figures in parentheses indicate number of patients.

Table 4 Correlation between plasma IL-6 concentrations and cumulative nitrogen balance postoperatively

	r	*P*
Total patients (38)	–0.2092	0.249
Ala-Gln group (20)	–0.6608	0.027[a]
Conv group (18)	0.033	0.919

[a]P<0.05 using pearson's correlation.

DISCUSSION

In this study, we analyzed plasma IL-2, IL-6, IL-8, and IFN-γ concentrations on postoperative days, because these cytokines are involved in the response to tissue injury[19,20]. IL-2 is produced by mitogen-stimulated T lymphocyte, and was originally isolated as a T-cell growth factor. IFN-γ promotes production of proinflammatory mediator such as IL-1 and TNF-α[19,20]. IL-1β, TNF-α, and IL-6 are major mediators of inflammation and acute phase response. We only measured IL-6 levels, because TNF-α and IL-1 have rarely been detected in the plasma of injured patients[20,21]. In this study, plasma IL-2 and IFN-γ were not detectable either. It is possible that the cytokines are bioactive in biologic fluid at levels well below the range of detectability by current immunoassays[20]. The effect of Gln supplementation on plasma IL-2 and IFN-γ cannot be found in this study.

IL-8 is a chemotactic and activating factor for immune response. Previous reports had shown that plasma IL-8 concentration were much higher in patients with sepsis compared with noninfectious shock, and associated with a fatal outcome[19]. Study by De Beaux *et al*.[17], showed that Gln-supplemented TPN reduces *in vitro* blood mononuclear cell IL-8 release in acute pancreatitis. In this study, no significant differences in plasma IL-8 concentrations were observed between the postoperative d 1 and 6. Also, there were no differences in IL-8 levels between the two groups on postoperative days. This result indicated that Gln administration did not influence the changes of IL-8 in surgical patients. Since IL-8 is associated with clinical,

biochemical, and inflammatory markers of sepsis[22,23], it is compatible to the fact that patients in this study were not complicated with infection, as shown in our previous report[11].

IL-6 is a multifunctional cytokine expressed by a variety of cells. Cruickshank *et al.*[21], reported that systemic response of IL-6 to surgical trauma increased with the severity of surgical insult. Elevation of IL-6 after trauma and elective surgery has been associated with infectious morbidity as well as mortality[24]. Foex and Shelly[25] suggest that IL-6 is a sensitive marker of tissue damage after surgery. In this study, we observed that plasma IL-6 concentrations significantly reduced after TPN administration for 6 d, regardless whether the patients were in Conv group or Ala-Gln group. This result might mean that TPN formula administration attenuated the inflammatory response of the surgical insult. The result also showed that plasma IL-6 levels on postoperative d 6 in the Ala-Gln group were significantly lower than the Conv group in patients with APACHE II$\leqslant$6, but not in patients with higher APACHE II scores. This finding suggests that in patients with lower disease severity, TPN supplemented with Gln had a significant favorable effect on reducing inflammatory response postoperatively. Studies have shown that after operation and trauma, Gln deprivation is observed and the severity of Gln deprivation is proportional to the severity of diseases[3,4]. It is possible that in patients with lower APACHE II score, the demand of Gln is much less than the patients with higher score, and Gln supplementation provides adequate substrate for the function of immune system, and thus reduced the inflammatory response of the surgical insult. Palmer *et al.*[4], showed that TPN supplemented with Gln had no effect on muscle and plasma biochemical changes in critically ill patients. Since this study was a post hoc investigation, whether higher dose of Gln may have beneficial effect on reducing IL-6 concentrations in patients with higher disease severity needs to be clarified.

In this study, we observed a negative correlation between plasma IL-6 and cumulative nitrogen balance postoperatively in the Ala-Gln group, whereas such correlation was not found when data of all patients were pooled and in the Conv group. This finding may indicate that the severity of protein catabolism cannot be predicted by plasma IL-6 level, possibly because the variation of nitrogen loss and plasma IL-6 levels was wide among individual surgical patient. However, Gln administration may consistently improve nitrogen balance and reduced plasma IL-6, and thereby a negative correlation between cumulative nitrogen balance and plasma IL-6 was found. Since nitrogen balance represents a complicated net result of protein catabolism and synthesis in the body, whether Gln play roles in modulating the relationship between IL-6 and protein metabolism required further investigation.

In summary, this study showed that TPN supplemented with Gln dipeptide had no significant influence on plasma IL-8 levels after surgery. However, Gln supplementation had a beneficial effect on decreasing systemic IL-6 production after surgery in patients with low admission illness severity, and lower plasma IL-6 may improve nitrogen balance in patients with abdominal surgery when Gln was administered.

ACKNOWLEDGMENTS

The authors thank Ms. Irene Cheng and Ms. Hui-Jen Huang for their help in collecting data and blood samples. We thank Frensenius AG (Bad Homburg, Germany) for supporting and supplying Gln dipeptide.

REFERENCES

1 **Bergstrom J,** Furst P, Noree LO, Vinnars E. Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol* 1974; **36**: 693-697
2 **Smith RJ,** Willmore DW. Glutamine nutrition and requirements. *J Parenter Enter Nutr* 1990; **14**: 94S-99S
3 **Parry-Billings M,** Baigrie RJ, Lamont PM, Morris P, Newsholme EA. Effects of major and minor surgery on plasma glutamine and cytokine levels. *Arch Surg* 1992; **127**: 1237-1240
4 **Palmer TEA,** Griffiths RD, Jones C. Effect of parenteral L-glutamine on muscle in the very severely ill. *Nutrition* 1996; **12**: 316-320
5 **Willmore DW,** Shabert JK. Role of glutamine in immunologic responses. *Nutrition* 1998; **14**: 618-626
6 **De-Souza DA,** Greene LJ. Phamacological nutrition after burn injury. *J Nutr* 1998; **128**: 797-803
7 **Quan ZF,** Yang C, Li N, Li JS. Effect of glutamine on change in early postoperative intestinal permeability and its relation to systemic inflammatory response. *World J Gastroenterol* 2004; **10**: 1992-1994
8 **Li JY,** Lu Y, Hu S, Sun D, Yao YM. Preventive effect of glutamine on intestinal barrier dysfunction induced by severe trauma. *World J Gastroenterol* 2002; **8**: 168-171
9 **Jiang ZM,** Cao JD, Zhu XG, Zhao WX, Yu JC, Ma EL, Wang XR, Zhu MW, Shu H, Liu YW. The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in postoperative patients: a randomized, double-blind, controlled study of 120 patients. *J Parenter Enter Nutr* 1999; **23**: S62-S66
10 **Morlion BJ,** Stehle P, Wachtler P, Siedhoff HP, Koller M, Konig W, Furst P, Puchstein C. Total parenteral nutrition with glutamine dipeptide after major abdominal surgery. *Ann Surg* 1998; **227**: 302-308
11 **Lin MT,** Kung SP, Yeh SL, Lin C, Lin TH, Chen KH, Liaw KY, Lee PH, Chang KJ, Chen WJ. Glutamine-supplemented total parenteral nutrition on nitrogen economy depends on severity of diseases in surgical patients. *Clin Nutr* 2002; **21**: 213-218
12 **Heberer M,** Babst R, Juretic A, Gross T, Horig H, Harder F, Spagnoli GC. Role of glutamine in the immune response in critical illness. *Nutrition* 1996; **12**: S71-S72
13 **Lennard TW,** Shenton BK, Borzotta A, Donnelly PK, White M, Gerrie LM, Proud G, Taylor RM. The influence of surgical operations on components of human immune system. *Br J Surg* 1985; **72**: 771-776
14 **Parry-Billings M,** Evans J, Calder PC, Newsholme EA. Does glutamine contribute to immunosuppression after major burns? *Lancet* 1990; **336**: 523-525
15 **Rohde T,** Maclean DA, Klarlund Pedersen B. Glutamine, lymphocyte proliferation and cytokine production. *Scand J Immunol* 1996; **44**: 648-650
16 **Yaqoob P,** Calder PC. Glutamine requirement of proliferating T lymphocytes. *Nutrition* 1997; **13**: 646-651
17 **De Beaux AC,** O'riordain MG, Ross JA, Jodozi L, Carter DC, Fearon KC. Glutamine-supplemented total parenteral nutrition reduces blood mononuclear cell interleukin-8 release in severe acute pancreatitis. *Nutrition* 1998; **14**: 261-265
18 **Dellinger EP.** Use of scoring systems to assess patients with surgical sepsis. *Surg Clin North Am* 1988; **68**: 123-145
19 **Ertel W,** Morrison MH, Wang P, Zheng F, Ayala A, Chaudry IH. The complex of cytokines in sepsis. *Ann Surg* 1991; **214**: 141-148

20 **Fong Y,** Moldawer LL, Shires GT, Lowry SF. The biologic characteristics of cytokines and their implication in surgical patients. *Surg Gynecol Obstet* 1990; **170**: 363-378
21 **Cruickshank AM,** Fraser WD, Burns HJG, Van Dame J, Shenkin A. Response of serum interkeulin-6 in patients undergoing elective surgery in varing intensity. *Clin Sci* 1990; **79**: 161-165
22 **Marty C,** Misset B, Tamion F, Fitting C, Carlet J, Cavaillon JM. Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. *Crit Care Med* 1994; **22**: 673-679
23 **Baigrie RJ,** Lamont PM, Kwiatkowski D, Dallman MJ, Morris PJ. Systemic cytokine response after major surgery. *Br J Surg* 1992; **79**: 757-760
24 **Biffl WL,** Moore EE, Moore FA, Peterson VM. Interleukin-6 in the injured patient: marker of injury or mediator of inflammation? *Ann Surg* 1996; **224**: 647-664
25 **Foex BA,** Shelly MP. The cytokine response to critical illness. *J Accid Emerg Med* 1996; **13**: 154-162

Science Editor Guo SY **Language Editor** Elsevier HK