附件一

行政院國家科學委員會補助專題研究計畫 ☑成果報告 □期中進度報告

（計畫名稱）

Gln對胃癌手術及胃癌細胞凋亡影響

計畫類別：☑ 個別型計畫　□ 整合型計畫

計畫編號：NSC91－2314－B－002－245

執行期間：　91 年 08 月 01 日至　92 年 07 月 31 日

計畫主持人：林明燦

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計畫參與人員：郭明良

成果報告類型(依經費核定清單規定繳交)：☑精簡報告　□完整報告

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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執行單位：台大醫院 外科

中　華　民　國　93　年　02　月 12 日

(二)中英文摘要及關鍵字(Keyword)：

I、中文摘要

關鍵詞：麩胺 (Glutamine)，全靜脈營養(Total Parenteral Nutrition，TPN)，胃癌(Gastric Cancer)，手術(Surgery)，細胞激素(Cytokine)，IL-6，細胞凋亡(Apoptosis)。

全靜脈營養(Total Parenteral Nutrition，TPN)是外科學歷史上對整個醫學的一項劃時代重大貢獻。只有在良好的營養醫療及支持(Nutrition therapy and nutrition support)之下，才能讓外科醫師積極進行胃癌重大手術，達成治療目標，挽救病人性命，並且減少手術的併發症發生，甚至有機會能減少癌症的再發。然而，傳統型的全靜脈營養(Conventional Standard TPN, S-TPN)缺乏很多的營養素，因此其中最重要的營養素之一是麩胺酸(Glutamine，Gln)。因此，傳統型的全靜脈營養必需加以改良，讓全靜脈營養更趨完善。然而，Gln-TPN 對人體的影響機制所知有限，特別是 Gln-TPN 對腫瘤方面影響的瞭解更是幾乎沒有。

隨著外科手術技術與周術期的照料(Perioperative care)的日益進步，愈來愈多的病人接受胃癌手術的治療。手術是目前唯一可以有效治療胃癌的治療方式。因此，積極的胃癌手術方式愈來愈講究與大膽，包括 D2 乃至於 D4 的淋巴廓清術，合併器官切除等等。現代的外科醫師，一方面要精進積極(Aggressive)與擴清性(Radical)的手術技術。更應該瞭解大手術(Major operation)對病人所產生的生理變化，包括對腫瘤生長或擴散的影響。

吾人以前的研究發現外科侵襲時，特別是大手術(Major Operation)，例如胃癌手術，會引發大量細胞激素(Cytokine)反應。適當的細胞激素分泌可提升病人免疫力與增進蛋白質代謝，加速病人的復原。但當這些細胞激素全身性過度反應(Systemic Exaggerated Response)時反而傷害病人，造成多器官功能不全(Multiple Organ Failure)。局部(Local)與全身性(Systemic)細胞激素之反應，在身體對抗外科手術壓力時，扮演之角色可能不同，然而這種胃癌周術期局部與全身細胞激素反應並不清楚。與普通簡單手術相比較，積極的根治性(Radical)胃癌手術可能引發過剩的細胞激素 IL-6 反應，然而詳細的反應並不清楚。

添加麩胺酸全靜脈營養(Gln-supplemented TPN，Gln-TPN)是最近發展的改良型全靜脈營養法之一。Gln 是腸細胞以及免疫細胞重要的營養素。研究顯示，Gln 可防止腸黏膜萎縮並增進免疫細胞機能。然而，以前關於 Glutamine 靜脈營養投與，幾乎沒有針對人體細胞激素影響及癌細胞生長與凋亡作研究。添加 Gln 靜脈營養，如何影響癌症病人手術術後的細胞激素反應，目前並不十分清楚。因此，本研究第一部分乃針對胃癌外科手術病患，包括 D2，D4，LN dissection，在術後給予傳統型 TPN(S-TPN)與 Glutamine 添加 TPN(Gln-TPN)，並比較二者 IL-6 的反應差異。與 S-TPN 為 isocaloric (30 kcal/kg/D)且 isonitrogen (1.5 gN/kg/D)。以 ELISA 方法測定手術前、後第 7 日局部引流液及血中的各細胞激素的濃度。預期 Glutamine TPN 營養投與對術後病人細胞激素可能有重要的影響。本研究第一部分結果可瞭解術後 Gln-TPN 是否對手術病人有較佳的細胞激素 IL-6 調節效果，並期能達到減低術後併發症的目的。

另一方面，吾人的研究報告指出 IL-6 會影響腫瘤的生長與細胞凋亡。因此，Gln 可能會藉由 IL-6 調節影響胃癌細胞的生長，本研究的 In Vitro Study 乃設計 Gln 不同濃度(0, 1, 2, 3 mM)在 IL-6 (0, 20, 40, and 80 ng/ml)作用下，對胃癌細胞凋亡(Apoptosis)的影響。

本研究可能發現與貢獻：

1. 確定 Gln-TPN 如何影響胃癌病患手術後 IL-6 反應。
2. 確定不同濃度 Gln 在 IL-6 作用下，是否會刺激胃癌細胞的生長，是否會抑制細胞凋亡。
3. 將來展望：Gln-TPN 可能對胃癌手術的病人有調節 IL-6 作用。Gln 可能經由 IL-6 作用調控癌細胞的細胞凋亡。因此，Gln-TPN 一方面可能讓病人安全的渡過手術期，另一方面期待減少胃癌的擴散與再發，是將來外科領域的研究發展重點。

II、英文摘要

Keywords：Glutamine，Total Parenteral Nutrition (TPN)，Cytokine，IL-6，Gastric Cancer，Surgery，Apoptosis。

Total parenteral nutrition (TPN) is a milestone contribution to the surgical field. TPN is a life-saving modality and has improved the perioperative mortality and morbidity for patients with malnutrition undergoing surgery, espically in paitents with gastric cancer. Surgeons are encouraged to undertake aggressive radical surgery for gastric cancer only with good nutritional support. However, conventional TPN lacks many important nutrients, including glutamine. Glutamine is the important nutrients for enterocytes, immune cells, and cancer cell. Glutamine supplemented TPN may enhance patient immunity, and may be the advancement of TPN in 21st century. However, the detailed mechanisms of Gln-TPN effects on gastric cancer surgery are unknown.

As surgical skill and perioperative care progress, surgeons are more aggressive in doing surgery for gastric cancers to obtain good treatment result. Radical surgery lymph node dissection D2, D4 may cause the more stressful insult to the patients, as compared with conventional conservative surgery. However, physiological responses of patients to such stressful operation are not well understood. Recent studies revealed that cytokines, especially IL-6, are deeply involved in response to surgical stress. Local and circulatory (systemic) cytokine may have different functions in the patients receiving surgery. Local cytokine may involve the effect on immune function and wound healing process after surgery. Systemic hyperresponse of some cytokines may have detrimental actions to induce remote organ injury. However, characteristics of local and systemic IL-6 dynamics influenced by Gln-TPN in the patients with gastric cancer during perioperative period were not clarified. Therefore, first purpose of this study is to determine Gln-TPN effects on postoperative IL-6 response to gastric cancer surgery. Factors influencing the IL-6 production in gastric cancer surgery are not well studied. Extended lymph dissection may be indicated for advanced gastric cancer, but may also cause grave tissue injury and stress to the patients.

Our previous studies have investigated the influences of glutamine on the nitrogen balance. However, it is unclear whether Gln-TPN influence cytokine production in patients after gastric cancer surgery. This study is designed to investigate whether Gln-TPN will influence IL-6 production in patient following surgery for gastric cancer. Patients with gastric cancer receive, randomly, standard total parenteral nutrition (S-TPN group, n=20) or Gln-TPN (Gln-TPN group, n=20) after the operation. The nutrition fluid to both groups are isocaloric (30 kcal/kg/D) and isonitrogenic (1.5 gN/kg/D) between two groups. Gln-TPN group received 30% Gln in total amino acids. Samples of venous blood are collected on preoperative day (Pre), post-operative day 7 (POD7). Levels of IL-6 in plasma are determined. The characteristics of patients and lymph node dissection (D2 and D4) in the both groups are comparable.

Gln-TPN supply might have effect modulate cytokines responses after surgery. Gln-TPN might provide better regulation of cytokine responses after surgery than standard parenteral nutrition. After analyse the clinical results, we will test if patients receiving Gln-TPN might have less postoperative complications than patients receiving standard TPN.

IL-6 has been reported to affect various kinds of tumor behavior, including tumor

proliferation, differentiation, invasion et al. Gln may have effects on modulating patient IL-6 response. However, Gln's actions under IL-6 on gastric cancer growth have not been well studied. *In vitro* study of this investigation is designed to evaluate the Gln action under IL-6 on apoptosis of gastric cancer cells. The action of additional Gln (0, 1, 2, 3 mM) under IL-6 (0, 20 40, and 80 ng/ml) in the culture medium on the gastric cancer apoptosis will be determined using flowcytometry and gel electrophoresis.

This investigation can provide information of Gln-TPN's effect on perioperative local and systemic IL-6 response in gastric cancer surgery. It will be determined whether perioperative IL-6 affects cancer recurrence and patient survival. *In vitro*, Gln's action on gastric cancer cell apoptosis will be determined. Modification of IL-6 response by Gln-TPN may be anticipated for nutritional therapy for patient undergoing gastric cancer.

(三)報告內容

見附件（論文抽印本）

(四)計畫成果自評部分

1. *In vivo* 臨床上添加 Gln 全靜脈營養(Gln-TPN)為國內第一次的臨床試驗，並為亞洲除日本以外第一個成功的試驗。Gln-TPN 可顯著改善胃癌手術病患的負氮平衡及提高免疫力，甚具學術研究與臨床價值。
2. 研究內容與原計畫相符成度甚高，達成主要目標，特別是人體試驗與臨床貢獻上。但 *In vitro* 部分，因 Gln 添加對胃癌細胞的凋亡作用，較無顯著現象，仍在調整實驗環境試驗中。

Reprinted from

Clinical Nutrition

Clinical Nutrition (2002) **21(3)**: 213–218

doi:10.1054/clnu.2001.0528, available online at http://www.idealibrary.com on IDEAL

ORIGINAL ARTICLE

The effect of glutamine-supplemented total parenteral nutrition on nitrogen economy depends on severity of diseases in surgical patients

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Clinical Nutrition (2002) 21(3): 213–218
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doi:10.1054/clnu.2001.0528, available online at http://www.idealibrary.com on IDEAL

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Abstract—Background: Gln is an important substrate for enterocyte and rapid proliferation cells. Studies have shown that parenteral supplementation of Gln maintains the intracellular Gln pool, improves nitrogen balance and shortens hospital stay. However, some studies showed Gln-supplemented TPN had no effect on restoring the Gln pool in critically ill patients. *Objective:* To evaluate the effect of glutamine (Gln) dipeptide supplementation of total parenteral nutrition (TPN) on postoperative nitrogen balance and immune response of patients undergoing surgery. *Methods:* This study is a prospective, randomized double-blind clinical trial. APACHE II score and TISS were used to evaluate the patients after admission. Forty-eight patients with major abdominal surgery were allocated to two groups to receive isonitrogenous (0.228 g nitrogen/kg/day) and isoenergetic (30 kcal/kg/day) TPN for 6 days. Two groups (Conv and Ala-Gln) were further divided to high (APACHE ≥ 6) and low (APACHE <6) groups. Control group (Conv) received 1.5 g amino acids/kg/day, whereas the Ala-Gln group received 0.972 g amino acids/kg/day and 0.417 g of L-alanyl-L-glutamine (Ala-Gln)/kg/day. Blood samples were collected on day 1 and day 6 after surgery for plasma amino acid and CD4, CD8 cell and T lymphocyte analysis. Cumulative nitrogen balance were also measured on day 2, 3, 4, 5 postoperatively. *Results:* Although there was a tendency to have better cumulative nitrogen balance on the postoperative days in the Ala-Gln group, no significant difference was observed between two groups. However, a better significant cumulative nitrogen balance was observed on the 2nd, 3rd and 5th postoperative day in the Ala-Gln group than in the Conv group in patients with APACHE II <6, whereas no significant difference was noted in patients with APACHE II ≥ 6. No difference in urine 3-methylhistidine excretion were observed between the 2 groups. Patients in the Ala-Gln group had significant higher T lymphocyte and CD4 cells than did those in the Conv group. *Conclusion:* TPN supplemented with Gln dipeptide had beneficial effect on enhancing the immune response. However, the effect of Ala-Gln administration on improving nitrogen economy was only observed in patients with low APACHE II scores. These results may indicate that Gln required for reversing the catabolic condition may depend on the characteristics and severity of the diseases. © 2002 Published by Elsevier Science Ltd.

Key words: glutamine; glutamine dipeptides; abdominal surgery

Introduction

Surgical trauma induces an altered protein metabolism that is characterized by negative nitrogen balance and changes in the pattern of plasma-free amino acids (1, 2). Glutamine (Gln) is the most abundant free amino acid in plasma and the tissue pool (3). It is an important substrate for enterocyte and other rapidly proliferating cells (4). Numerous studies have established that hypercatabolic and hypermetabolic states are associated with profound Gln deprivation (5–8). The extent and duration of the Gln depletion are proportional to the severity of patient illness. In critically illness or during major surgery, muscle and lung Gln effluxes are accelerated to provide substrate for the gut, immune cells and kidney, which may consequently result in profound decline in muscle-free Gln concentrations (8, 9). Total parenteral nutrition (TPN) is commonly used in the treatment of critically ill patients. Since commercially available amino acid solutions do not contain Gln because of its instability in aqueous solution, conventional TPN does not prevent stress induced Gln depletion. Some previous studies indicated that administration of free Gln or Gln-containing depeptides improve nitrogen balance and maintain intracellular Gln pool, enhance lymphocyte recovery, cysteinyl-leukotriene generation, and shortened hospital stay in surgical patients (10, 11). However, a study by Palmer et al. (12) showed that TPN supplemented with crystalline L-Gln appears to have no effect on muscle

and plasma biochemical changes in critically ill patients. Also, Karner et al. (13) demonstrated that TPN supplemented with a solution containing Gln either in free or in dipeptide form did not restore the intracellular Gln pool in skeletal muscle in dogs with sepsis. The discrepancies between these studies may result from the duration and dosage of Gln administered. Besides, the characteristics and severity of diseases may also play important roles in influencing the efficacy of Gln supplementation. In this study, we administered a Gln dipeptide to patients, postoperatively in order to investigate the effect of synthetic Gln supplementation on nitrogen economy and immune states. Further, we evaluated whether the Gln effect are dependent on the severity of stress of the patients after surgery.

Patients and methods

This study comprised patients who had no major metabolic, circulatory and renal diseases and were all admitted for elective abdominal surgery. No emergency cases were included. Chronic health evaluation (APACHE II) score and Therapeutic Intervention Scoring System (TISS) were evaluated after admission. Patients with APACHE II between 2–10 and TISS > 10 were included. A total of 48 patients (28 males, 20 females, age range 40–82 years, mean 66 years) were enrolled from 2 centers. These patients were randomly allocated to either a test group or a control group. Demography for all patients was summarized in Table 1. This study was approved by the ethical committee of the College of Medicine, National Taiwan University, and Veterans General Hospital, Taipei, Taiwan. The purpose of the experiment was explained to the patients, and informed consent of the patients were obtained before the study started.

All pateints received TPN given continuously during six days with 0.228g/kg nitrogen (isonitrogenous) and 30 kcal/kg daily (isocaloric) postoperatively. 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/d. Patients of the test group (Ala-Gln group, $n=25$) received 0.972 g amino acid/kg/d supplemented with 0.417 g/kg/d L-Alanyl-L-Glutamine which provide 0.28 g/kg/d glutamine (Dipeptiven, Fresenius Kabi, Bad Homburg, Germany). The nonprotein calories were given as glucose and fat in a ratio of 70 : 30. The Gln containing solutions were prepared by the clinical pharmacist under aseptic condition and were adjusted to the weight of each individual patient. The amino aicds and glucose mixture with other electrolytes, vitamins and trace elements were administered through a central venous catheter. Fat emulsion (Lipovenos 20%, Fresenius AG, Germany) 0.8 g/kg/d was given during 5 hours through a separate peripheral or central venous line in both groups. Half dose was administered on the first day, and the entire dose was given thereafter during the remaining days. Since this study was a randomized, controlled double-blind clinical trial, test and control solutions were packed at the same size and packaging materials have the same appearance. Neither the patient nor the investigator knew if the applied TPN regimen were with or without Ala-Gln.

Urine and drainage fluid were collected throughout the study. Nonprotein nitrogen content was measured by a colorimetric method. Nitrogen loss in stool and from the skin were estimated as 2.0 g/day in the study. Cumulative nitrogen balance was calculated from postoperative day 2 to day 5.

Routine blood chemistry was measured before surgery. The blood samples were also obtained on postoperative day 1 before TPN infusion and on day 6 after TPN administration for routine laboratory test, and analysis of plasma-free amino acid as well as the population of T lymphocyte. Routine biochemical measurements were assessed by standard methods. Plasma free amino acid and urine 3-methylhistidine were determined by the standard ninhydrin technology (Model 6300, Beckman Instrument, Palo Alto, CA, USA) after deproteinization of the plasma or urine with 50% salicylic acid (14). Flow cytometry was used to determine the proportions of CD4+ and CD8+ lymphocytes in fresh blood. One hundred microliters of blood was incubated for 15 min at +4°C with 10 µl of

Table 1 Demographic data of patients

	Ala-GLN		Control	
	APACHE < 6	APACHE ≥ 6	APACHE < 6	APACHE ≥ 6
Age (years)	61.7 ± 11.2	70.1 ± 6.0	64.5 ± 7.6	70.6 ± 8.7
Weight (kg)	57.7 ± 8.6	56.3 ± 10.1	55 3 ± 8.6	59.8 ± 12.0
Height (cm)	160.45 ± 6.7	156.0 ± 6.1	158.8 ± 8.0	161.3 ± 9.0
Male, Female	4/6	10/5	6/5	8/4
Diagnosis:				
Gastric Ca	6	12	6	8
Pancreas Ca	3	1	2	2
Colon Ca	0	1	2	1
Hepatoma	1	0	1	0
Adenocarcinoma of duodenum	0	0	0	1
Rectal adenocarcinoma	0	1	0	0

phycoerythrin-conjugated (PE) mouse monoclonal anti-human CD4+ (0.48 μg/ml) and phycoerythrin-conjugated (PE) mouse anti-human CD8+ (0.3 μg/ml) (Immunotech, Marseille, France). Red blood cells were then hemolysed with lysing buffer. Fluorescence data were collected on 5×10^4 viable cells and analyzed by flow cytometry (Coulter, Miami, FL, USA).

Statistics

The data are expressed as mean ± SEM, repeated measure analysis of variance (ANOVA) were used to compare the treatment differences at each day, and *t*-test was used for the difference between the 2 groups. A P value <0.05 was considered significant. A normal probability plot was used for detecting the model residuals in the analysis of variance. Since moderate departures from normality are usually of little concern in the fixed effects analysis of variance, and the F-test was only slightly affected, the analysis of variance was robust to the normality assumption.

Results

No adverse reactions were found after the peptide-containing solution was administered. The patients had normal plasma biochemistry values at baseline and on day 6 after operation in either Conv or Ala-Gln groups.

Although there was a tendency to have better cumulative nitrogen balance on the postoperative days in the Ala-Gln group, no significant difference was observed between the 2 groups. However, significant better cumulative nitrogen balance was observed on the 2nd, 3rd and 5th postoperative day than those in the Conv group in patients with APACHE II <6, whereas no significant difference was noted in patients with higher APACHE II score (Fig. 1). There was no difference in plasma Gln concentrations after TPN administration between the 2 groups. However, plasma Gln concentrations in all the 10 patients ($n=10$) with APACHE II <6 were elevated, whereas plasma Gln concentration in 8 patients in the Conv group ($n=11$) were decreased on postoperative day 6 as compared with day 1. The changes between postoperative day 1 and 6 were significantly different between the 2 groups in the APACHE <6. This phenomenon was not observed in the patients with APACHE II ≥ 6 (Table 2). In patients with APACHE II <6, urine 3-methylhistidine concentrations on the postoperative day 6 were significantly lower than day 1, in both Ala-Gln and Conv groups. The reduction of urine 3-methylhistidine excretion after infusing TPN for 6 days was not obvious in patients with APACHE II ≥ 6 (Table 3). Patients in the Ala-Gln group had higher proportion of T lymphocyte and CD4 cells than did those in the Conv group (Table 4). The proportion of CD4 cells was greater after TPN

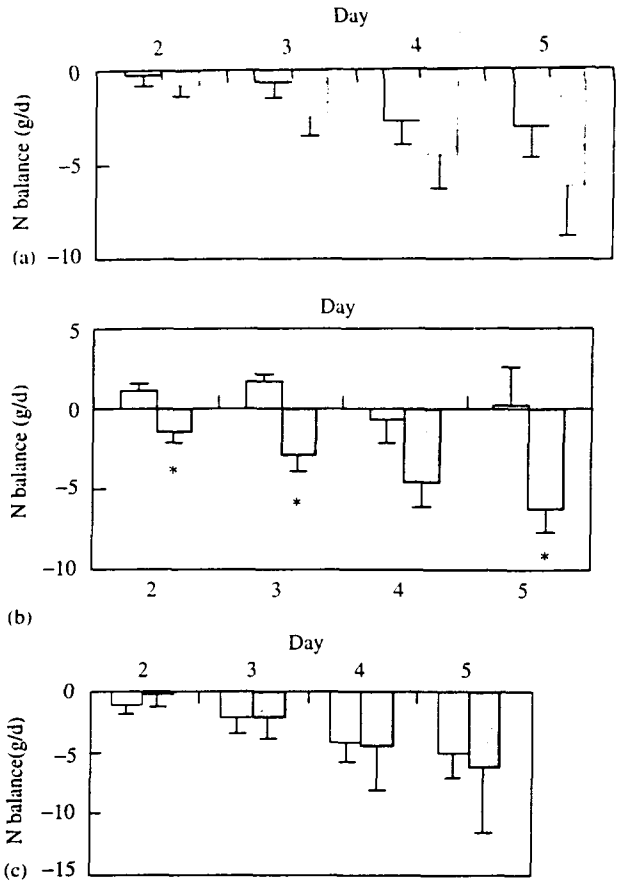

Fig. 1 Cumulative nitrogen balance in patients receiving TPN supplemented with L-alanyl-L-glutamine (Ala-Gln) or conventional (Conv) TPN. (A) No statistically significant difference was observed between the 2 groups on different postoperative days in all patients. (B) Ala-Gln group had better cumulative nitrogen balance on day 2, 4, 5 postoperatively in patients with APACHE II <6. (C) No significant difference was observed between the 2 groups on different postoperative days in patients with APACHE II ≥ 6. Mean ± SEM, significant between 2 groups, $^*P<0.05$. □ Ala-Gln; □ Conv.

administration for 6 days. Adverse effects were listed in Table 5. There was no mortality and no significant different adverse effects or morbidity between the two groups.

Discussion

Gln is a very important amino acid because it is the primary nitrogen donor in DNA synthesis at the cellular level and is involved in interorgan nitrogen transport and bicarbonate generation in the kidney (15, 16). When metabolic demand for Gln exceeds available supply as occurs in critically illness, a relative deficiency results. After operation and trauma, a negative nitrogen balance is observed with Gln deprivation in muscle mass. This alteration is not prevented by administrating conventional TPN after operation. Studies by Pettersson et al. (17) and Hammarqvist et al. (5, 10) showed that TPN with Gln may spare free Gln in muscle, improve

Table 2 Plasma glutamine concentration on day 1 postoperatively and on day 6 after TPN administration

	Conv	Ala-Gln
All patients	(23)	(25)
Day 1[#]	471.6±18.1	531.7±49.8
Day 6	485.0±20.2	523.5±29.2
Changes (day 6-day 1)	13.2±31.1	−16.8±32.9
APACHE II <6	(11)	(10)
Day 1	499.9±30.5	512.2±36.7
Day 6	506.3±29.7	567.9±53.6
Changes (day 6-day 1)	6.2±30.2	99.6±42.5*
APACHE II ≥6	(12)	(15)
Day 1	443.3±17.1	540.1±70.2
Day 6	461.5±26.6	502.9±34.7
Changes (day 6-day 1)	24.5±27.2	3.5±34.7

[#]Day 1 represent the data obtained before TPN administration.
Figures in parentheses indicate number of patients.
*Significantly different from the Conv group ($P<0.03$).

Table 3 Urine 3-methylhistidine concentrations on day 1 postoperatively and on day 6 after TPN administration

	Conv	Ala-Gln
All patients	(23)	(25)
Day 1[#]	136.1±20.0	113.5±13.9
Day 6	82.3±8.8	72.0±10.8
Changes (day 6-day 1)	−59.0±15.3	−49.5±18.7
APACHE II <6	(11)	(10)
Day 1	136.7±26.7	125.1±21.1
Day 6	71.8±7.7*	60.8±16.4*
Changes (day 6-day 1)	−64.8±25.6	−66.8±15.3
APACHE II ≥6	(12)	(15)
Day 1	136.0±24.9	109.1±15.8
Day 6	73.3±12.4	68.2±10.3
Changes (day 6-day 1)	−52.6±21.0	−37.9±21.4

[#]Day 1 represent the data obtained before TPN administration.
*Significantly different from day 1.
Figures in parentheses indicate number of patients.

Table 4 Blood T lymphocyte, CD4, CD8 cell and total lymphocyte count in 2 TPN groups on day 1 postoperatively and day 6 after TPN administration

	Conv	Ala-Gln
Day 1	(23)	(25)
T lymphocyte (%)	57.09±2.52	65.95±2.18*
CD4 (%)	34.68±1.91	36.84±1.89
CD8 (%)	20.87±9.41	25.06±1.94
Total Lymphocyte ($\times 10^3$/ml)	107.33±9.89	107.07±9.18
Day 6	(23)	(25)
T lymphocyte (%)	62.0±2.62	69.72±2.85*
CD4 (%)	41.76±2.48[#]	46.69±2.32*[#]
CD8 (%)	18.53±1.75	20.38±1.61
Total Lymphocyte ($\times 10^3$/ml)	130.6±15.29	114.19±10.68

*Significantly different from Conv group.
[#]Significantly different from the corresponding group on day 1 postoperatively.
Figures in parentheses indicate number of patients.

Table 5 Summary of adverse events

	Ala-Gln Total = 25		Conv Total = 23		
	N	(%)	N	(%)	*P*-value
COPD aggravated	0	(0.00)	1	(4.35)	0.479
Cath lost	2	(8.00)	1	(4.35)	1.000
Fever	3	(12.00)	0	(0.00)	0.235
Tachycardia	1	(4.00)	0	(0.00)	1.000
Pleural effusion	1	(4.00)	0	(0.00)	1.000
Hyperglycemia	3	(12.00)	2	(8.70)	1.000
Wound infection	0	(0.00)	0	(0.00)	1.000
Leakage	0	(0.00)	0	(0.00)	1.000
Mortality	0	(0.00)	0	(0.00)	1.000

P-value: Test for equality between treatments using Fisher's exact test.
COPD: Chronic obstructive pulmonary disease.
Fever: Only transit fever, without evidence of infection.
Pleural effusion: X-ray finding without symptoms.

nitrogen balance and attenuate postoperative muscle decline in protein synthesis in surgical patients. Also, a study by Ardawi et al. (18) has shown that addition of Gln to TPN enhanced muscle protein synthesis and decreased protein degradation in septic rats. Morlion et al. (11) demonstrated that Gln dipeptide supplementation in TPN resulted in maintenance of plasma Gln levels on the postoperative days and had beneficial effect on nitrogen economy in patients undergoing major surgery. In our study, almost 30% of the total amino acid was isonitrogenously substituted by Gln, which might have resulted in a lower amount of essential amino acid in the Ala-Gln group. However, this amount was comparable to the reports by others, and was thought to be adequate for protein anabolism of the surgical patients (10, 11, 19).

In the present study, cumulative nitrogen balance on the postoperative days did not differ signigicantly between Gln and conventional group. The separation into low and high APACHE groups was post hoc. However, it is interesting that a better cumulative nitrogen balance was observed in the Ala-Gln group than the Conv group in the patients with APACHE II <6. Further studies using at least four randomized groups will be anticipated to confirm the hypothesis. In the population of patients with lower APACHE II score, plasma Gln levels were significantly elevated in the Ala-Gln group than in the Conv group. These finding may indicate that the Gln-dipeptide-containing solution increased plasma Gln levels and improved nitrogen economy only in patients with lower disease severity. Previous studies have shown that 13–20 g Gln is generally administrated for a 60–70 kg patient after major injury or uncomplicated elective surgery (5, 10, 11, 17, 20). Palmer et al. (12) reported that TPN supplemented with 25 g crystalline L-Gln appeared inadequate to counteract the muscle and plasma biochemical changes in critically ill patients. Also, a study by Karner et al. (13) showed that a Gln supply equivalent to 26 g in a patient weighing 70 kg had no effect on intramuscular Gln levels in sepsis. It is suggested that patients with multiple injury, burns, sepsis, as well as following bone marrow transplantation may require higher dose of Gln (21, 22). In this study, about 17–20 g Gln for a 60–70 kg patient was administered. It is possible that this amount of Gln was not potent enough to reverse the catabolic condition in

severe diseases. Whether longer duration or larger dose would alter this state requires further investigation.

3-methylhistidine is an amino acid presents in the actin of skeletal muscle and the myosin of white fibers. Studies have confirmed that skeletal muscle mass is related to endogenous urinary 3-methylhistidine excretion (23). Although urinary 3-methylhistidine does not totally reflect muscle protein breakdown, it is an index of muscle protein catabolism in trauma (23, 24). The result in this study revealed that urine 3-methylhistidine excretion was significantly lower on day 6 compared to day 1 in patients with APACHE II <6, but not in patients with higher APACHE II scores. This finding indicated that in the patients with lower disease severity, TPN administration attenuated the muscle protein catabolism. However, compared with Conv solution, Gln supplementation had no favorable effect on reducing muscle protein breakdown in the present experimental condition. Since urinary 3-methylhistidine did not differ after TPN administration between the 2 groups, the beneficial effect on improving cumulative nitrogen balance after Ala-Gln administration may result from the anabolic effect of Gln on enhancing body protein synthesis.

Gln is an important substrate for rapid proliferating cells such as macrophages and lymphocytes (4, 25). Gln supplementation in the Ala-Gln group may effect on the immune system in patients with major surgery. Previous studies have shown that major operations and injuries result in a variable degree of immune dysfunction (26–28). Li et al. (29) demonstrated that, compared with chow-fed animals, conventional TPN depressed the CD4+/CD8+ ratio within the lamina propria, and that the depression was reversed with Gln supplementation of the TPN solution. A study by O'Riordain et al. (19) evaluated the effect of Gln supplemented TPN on T-cell response in patients undergoing elective colonic resection. They found that peripheral blood mononuclear cell isolated from patients with Gln supplementation had better T-cell response and DNA synthesis than the control TPN group. Also, Alverdy et al. (30) reported that addition of Gln to standard TPN solution maintained T cell populations at levels similar to those of chow-fed animals. In this study, the proportions of CD4+ helper-type cells and T lymphocyte were significantly higher in the Ala-Gln group than in the Conv group. Since the immune response to infection is rapid, the rate of Gln utilization might be high to provide optimal conditions for such response (31). It is possible that the priority of Gln in enhancing cellular immunity is superior to that for nitrogen metabolism.

In summary, the present study shows that TPN supplemented with Gln dipeptide had beneficial effect immune response postoperatively. The effect of Ala-Gln administration on increasing plasma Gln concentrations and improving nitrogen economy was only observed in patients with lower disease severity. Since the patients with APACHE II score ≥ 6 may have higher metabolic stress, the variation in plasma Gln levels and cumulative nitrogen balance were wide in the Ala-Gln group, and the favourable effect of Gln administration in nitrogen metabolism was not obvious. These results may indicate that the characteristics and severity of the diseases play roles in determining the amount of Gln required. Whether longer duration or higher doses of Gln dipeptide may have favorable effect on nitrogen economy and attenuate muscle protein breakdown in diseases with higher severity required further investigation.

Acknowledgements

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

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Submission date: 1 June 2001 *Accepted:* 14 November 2001