Journal of Parenteral and Enteral Nutrition

http://pen.sagepub.com/

Perioperative Oral Administration of Cystine and Theanine Enhances Recovery After Distal Gastrectomy : A Prospective Randomized Trial

Tomohiro Miyachi, Takashi Tsuchiya, Atsushi Oyama, Takahiro Tsuchiya, Naomi Abe, Atsuko Sato, Yasumasa Chiba, Shigekazu Kurihara, Tetsuro Shibakusa and Takashi Mikami

JPEN J Parenter Enteral Nutr published online 12 September 2012

DOI: 10.1177/0148607112458798

The online version of this article can be found at:

http://pen.sagepub.com/content/early/2012/09/11/0148607112458798

Published by:

SAGE

http://www.sagepublications.com

On behalf of:

The American Society for Parenteral & Enteral Nutrition

Original Communication

aspen LEADING THE SCIENCE AND PRACTICE OF CLINICAL NUTRITION
American Society for Parenteral and Enteral Nutrition

Perioperative Oral Administration of Cystine and Theanine Enhances Recovery After Distal Gastrectomy: A Prospective Randomized Trial

Journal of Parenteral and Enteral Nutrition
Volume XX Number X
Month 2012 1-8

DOI: 10.1177/0148607112458798
http://jpen.sagepub.com
hosted at
http://online.sagepub.com
SAGE

Tomohiro Miyachi, MD[1]; Takashi Tsuchiya, MD, PhD[1]; Atsushi Oyama, MD, PhD[1]; Takahiro Tsuchiya, MD[1]; Naomi Abe, RD[2]; Atsuko Sato, RD[2]; Yasumasa Chiba, MSc[3]; Shigekazu Kurihara, PhD[3]; Tetsuro Shibakusa, PhD[3]; and Takashi Mikami, BVSc[3]

Abstract

Background: It has been reported that cystine and theanine, amino acids related to glutathione synthesis, have immunomodulatory effects, such as suppressing inflammation after strenuous exercise. In this study, we examined the effects of oral administration of cystine and theanine during the perioperative period as a pilot study. *Methods*: Forty-three cases of distal gastrectomy for cancer conducted in our department were assigned to the cystine and theanine group (CT group) or to the placebo control group (P group), and a randomized, single-blind, parallel-group study was then performed. Cystine (700 mg) and theanine (280 mg) or a placebo was administered to participants for 10 continuous days (4 days before to 5 days after surgery). Changes in pre- and postoperative interleukin (IL)–6, C-reactive protein (CRP), albumin, white blood cell (WBC) count, neutrophil count, total lymphocyte count, resting energy expenditure (REE), and body temperature were compared and examined. *Results*: Ten patients were excluded, leaving 33 patients in the study. The CT group had significantly lower IL-6 values (postoperative day [POD] 4), CRP levels (POD 7), neutrophil counts (POD 4), and body temperatures (POD 5) than the P group ($P < .05$). In addition, REE in the P group peaked on day 1 (1.14 ± 0.16 [pre- and postoperative ratio]), whereas the CT group did not show any increase on POD 1 (0.99 ± 0.21, $P < .05$ vs P group). *Conclusions*: This study suggests that oral administration of cystine and theanine during the perioperative period may alleviate postgastrectomy inflammation and promote recovery after surgery. (*JPEN J Parenter Enteral Nutr*. XXXX;xx:xx-xx)

Keywords

nutrition support teams; amino acids; immunonutrition; oncology

Clinical Relevancy Statement

Appropriate regulation of inflammation during the perioperative period is important for achieving a stable postoperative course and early recovery and for reducing the malignancy relapse rate. For these reasons, an immune-modulating diet that includes anti-inflammatory substances such as ω-3 fatty acids has been used widely in clinical settings. The amino acids cystine and theanine are both involved in the synthesis of glutathione (GSH), a strong antioxidant, and there have been several reports that oral cystine and theanine enhance immunoreactions. This study was a pilot study to assess the effect of perioperatively administering oral cystine and theanine in gastric surgery patients and suggested that oral administration of these amino acids may reduce inflammation and promote recovery after gastric surgery.

Introduction

Preventing organ dysfunction and immune suppression by inhibiting excess inflammation is considered an important aspect of perioperative surgical management, and several studies have indicated that immunonutrition is effective for this purpose.[1,2] Administering the amino acids cystine and theanine has been shown to significantly suppress increases in C-reactive protein (CRP) levels and neutrophil counts and decreases in the number of lymphocytes after excessive exercise, resulting in a reduction in excessive inflammation and immune dysfunction; similar effects are expected to result from immunonutrition.[3] Cystine is a sulfur-containing amino acid that consists of 2 cysteine molecules joined by a disulfide bond.[4] Transporters on cell membranes (including those of neutrophils, macrophages, and dendritic cells) mediate the uptake of cystine into cells, and then thioredoxin rapidly reduces it to produce cysteine.[5] Theanine is an amino acid

From the [1]Department of Surgery, Sendai City Medical Center, Sendai, Japan; [2]Department of Nutritional Management, Sendai City Medical Center, Sendai, Japan; and [3]Research Institute for Health Fundamentals, Ajinomoto Co, Inc, Kanagawa, Japan.

Financial disclosure: none declared

Received for publication January 19, 2012; accepted for publication July 30, 2012.

Corresponding Author: Takashi Tsuchiya, MD, PhD, Department of Surgery, Sendai City Medical Center, 5-22-1 Tsurugaya, Miyagino-ku, Sendai, Miyagi 983-0824, Japan; e-mail: tsuchiya@openhp.or.jp.

found in green tea that is metabolized in the intestinal tract and liver to yield glutamic acid and ethylamine.[6,7] Cystine and theanine are both involved in synthesizing glutathione (GSH), which is a tripeptide consisting of glutamic acid, cysteine, and glycine, and it has been confirmed that hepatic GSH concentrations increase after cystine and theanine administration in mice.[4,8,9] The importance of GSH to the immune system has been reported previously. Besides having a strong antioxidant effect, GSH plays an important role, particularly in the maintenance and regulation of the thiol-redox status of the cell, and these appear to correlate with proliferation or differentiation of lymphocytes, macrophages, and dendritic cells.[10-15] Studies of patients receiving elective abdominal surgery have found that GSH levels in blood and skeletal muscle decrease by 10% and 42%, respectively, within 24 hours of surgery, and preventing this reduction in GSH may reduce immune suppression during the perioperative period.[16]

In this study, we postulated that cystine and theanine would reduce excessive inflammation and immune suppression during the perioperative periods of abdominal surgeries, which decreases GSH levels in blood and skeletal muscle.[16] We examined the effects of perioperatively administering cystine and theanine on the postoperative course of gastric cancer patients undergoing distal gastrectomies as a pilot study.

Methods

Patients

All patients who underwent distal gastrectomy for gastric cancer by our department between January 2009 and October 2010; who were between 40 and 75 years old; who did not have distant metastasis, undernutrition (serum albumin <3.5 g/dL), decline in performance status (≥2), or pyloric stenosis for cancer; and who agreed to participate were included in the study.

Consent and Ethics

This clinical study was approved by the institutional review board of our hospital, and informed consent was obtained from patients after the nature of the procedure had been explained.

Study Design

Patients were assigned to 1 of 2 groups using the sealed-envelope method and a randomized, single-blind, parallel-group study was performed. Either cystine (700 mg) and theanine (280 mg) or a placebo (950 mg cellulose and 30 mg glutamine) was orally administered to patients with a small amount of water from 4 days before to 5 days after surgery, including the day of the procedure. Both groups received postsurgical management that followed the clinical pathway used at our hospital (Figure 1).

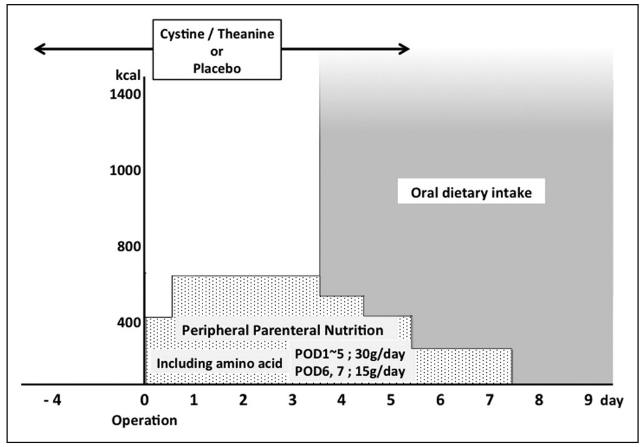

Figure 1. Perioperative management protocol. POD, postoperative day.

The dosage of cystine and theanine was determined based on human studies. Average daily intake of cystine from food has been reported to be 1000 mg/d with a maximum of 2200 mg/d.[17,18] From the viewpoint of safety, the amount of cystine was determined to be 700 mg. Experimental analysis revealed that a weight ratio of cystine to theanine of 5:2 was the best to achieve effective increases in GSH levels.[19] According to this ratio, the dosage of theanine was decided as 280 mg. These test substances are commercially available from Ajinomoto Co, Inc (Tokyo, Japan).

Operation Method

All patients were laparotomized by upper midline incision under general anesthesia with epidural anesthesia; distal gastrectomy and regional lymph node dissection were performed according to the guideline of the Japan Gastric Cancer Association, followed by reconstruction after the Billroth-I or Roux-en Y method.

Data Collection

Variables investigated included interleukin (IL)–6 (before surgery, immediately after surgery, and postoperative days [PODs] 1, 4, and 7); CRP, albumin, white blood cell (WBC) count, neutrophil count, and total lymphocyte count (measured before surgery and on PODs 1, 4, 7, and 14); resting energy expenditure (REE, measured before surgery and on PODs 1, 4, 5, 7, and 14); and maximum body temperature (before surgery, on the day of the procedure, and every day after surgery). Changes in these variables were examined and compared between the 2 groups. REE measurements were performed on an empty stomach in the late afternoon using a small expiration gas analyzer (MedGem; HealthTech, Inc, Golden, CO).

Table 1. Clinical Backgrounds and Demographics of Patients in the 2 Groups

	P Group (n = 18)	CT Group (n = 15)	*P* Value
Age, y	60.4 ± 7.8	60.3 ± 5.3	NS
Sex, M/F, No.	12/6	8/7	NS
Operation time, min	230 ± 38	211 ± 30	NS
Blood loss, g	283 ± 153	255 ± 148	NS
Degree of LN dissection, D1/D2, No.	12/6	10/5	NS
Type of reconstruction, B-I/R-Y, No.	15/3	12/3	NS
Stage (UICC/TNM), I/II/III/IV, No.	15/2/1/0	13/1/1/0	NS

All data are expressed as means ± SD unless otherwise indicated. B-I, Billroth-I method; CT, cystine and theanine; F, female; LN, lymph node; M, male; NS, not significant; P, placebo; R-Y, Roux-en-Y method; UICC, International Union Against Cancer.

Exclusion Criteria

Registered cases with excessive intraoperative hemorrhage (>600 mL), intraoperative complications, withdrawal of participation consent, and changes in surgical procedure were excluded from the study. The hemorrhage criterion was set as nearly twice the mean amount of bleeding during gastrectomy for cancer in our hospital. All criteria were set before starting the study.

Statistical Analyses

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 17 (SPSS, Inc, an IBM Company, Chicago, IL) or Sigma Stat3.1 statistical analysis software (Systat Software, Inc, Richmond, CA). In all analyses, $P < .05$ was taken to indicate significance. The Student *t* test and Mann-Whitney *U* test were used to compare results between the 2 groups. A 2-way analysis of variance (ANOVA) model and the χ^2 test were used to compare patient backgrounds between the 2 groups.

Results

Study Characteristics

A total of 43 cases were enrolled in this study: 22 cases in the cystine/theanine group (CT group) and 21 cases in the placebo group (P group). Of these cases, 10 were excluded due to the onset of herpes zoster before the procedure (n = 1), excessive intraoperative hemorrhage (n = 2), injury to the left hepatic artery during surgery (n = 1), changes in surgical procedure (n = 2), metastasis to the peritoneum confirmed during surgery (n = 1), or withdrawal of consent (n = 3). Therefore, the study population consisted of a total of 33 cases: 15 cases in the CT group and 18 cases in the P group. There were no significant differences in age, sex, amount of intraoperative bleeding, duration of surgery, degree of lymph node dissection and type of reconstruction, or course of disease progression (*International Union Against Cancer [UICC]/TNM Classification of Malignant Tumours*, seventh edition)[20] between the 2 groups (Table 1). All 33 cases in the study were managed using the clinical pathway; no cases deviated from the pathway, and no complications were observed after the procedure. No cases required blood transfusion.

Outcomes

IL-6 levels (measured values) in the 2 groups showed a peak immediately after surgery and then decreased; however, lower values were observed in the CT group during the study period (*P* = .052 on POD 1 and *P* = .058 on POD 7) than that in the P group, and a significantly lower value was observed on POD 4 in the CT group (7.96 ± 3.50 pg/mL) than that in the P group (13.86 ± 10.21 pg/mL) (*P* = .048) (Figure 2a).

In both groups, CRP levels (measured values) were highest on POD 1 and decreased thereafter, but this value in the CT group (1.55 ± 0.94 mg/dL) was significantly lower than that in the P group (2.62 ± 1.74 mg/dL) on POD 7 (*P* = .043) (Figure 2b).

Serum albumin levels (measured value) were lowest on POD 4 and subsequently increased. There were no significant differences between the 2 groups (data not shown).

Neutrophil count, neutrophil fraction, and total lymphocyte fraction (ratio relative to preoperative levels) all differed significantly between the 2 groups (Figures 3 and 4). Neutrophil count and neutrophil fraction were highest on POD 1 and tended to recover after POD 4. However, the CT group had lower values throughout the study period, and neutrophil count and neutrophil fraction were significantly different between the 2 groups on POD 4 and POD 7, respectively (*P* = .041 and *P* = .041) (Figures 3a, 4a). Total lymphocyte count began to decrease on POD 1, reached its lowest value on POD 4, and began to recover afterward; however, there were no significant differences between the 2 groups (Figure 3b). Total lymphocyte fraction in the CT group showed faster recovery after the decrease and was significantly different from the fraction in the P group on POD 7 (*P* = .027) (Figure 4b). Changes in WBC count were not significantly different between the 2 groups (data not shown).

Preoperative REEs were 1414 ± 201 kcal in the CT group and 1408 ± 241 kcal in the P group. After surgery, REE in the P group peaked on POD 1 (1609 ± 330 kcal, 1.14 ± 0.16

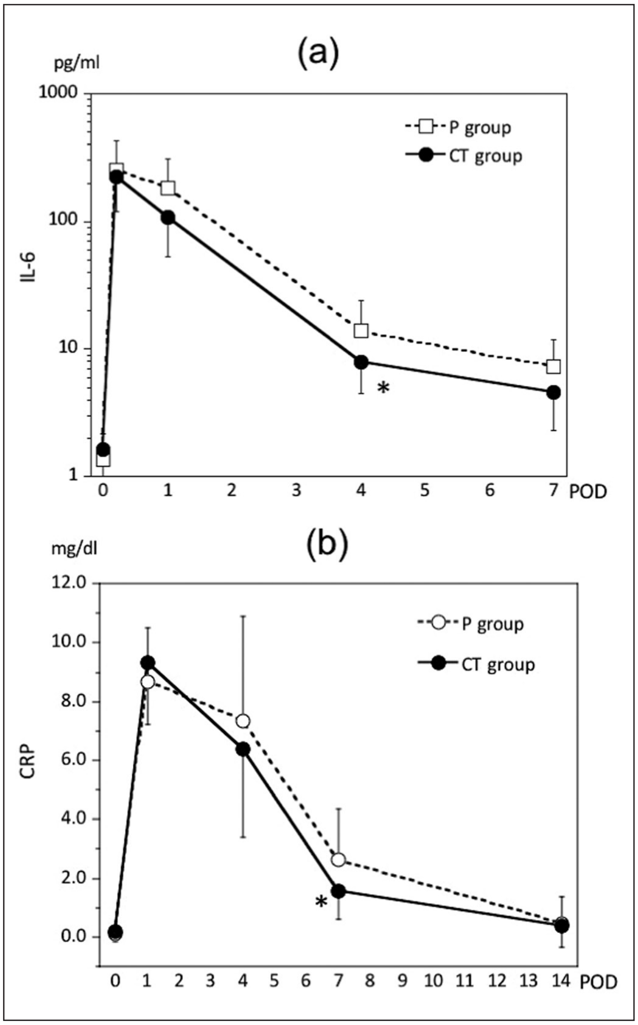

Figure 2. Serum interleukin (IL)–6 (a) and C-reactive protein (CRP) (b) profiles throughout the perioperative period. All data are expressed as the mean ± SD. CT, cystine and theanine; POD, postoperative day. *$P < .05$ vs values in the placebo (P) group.

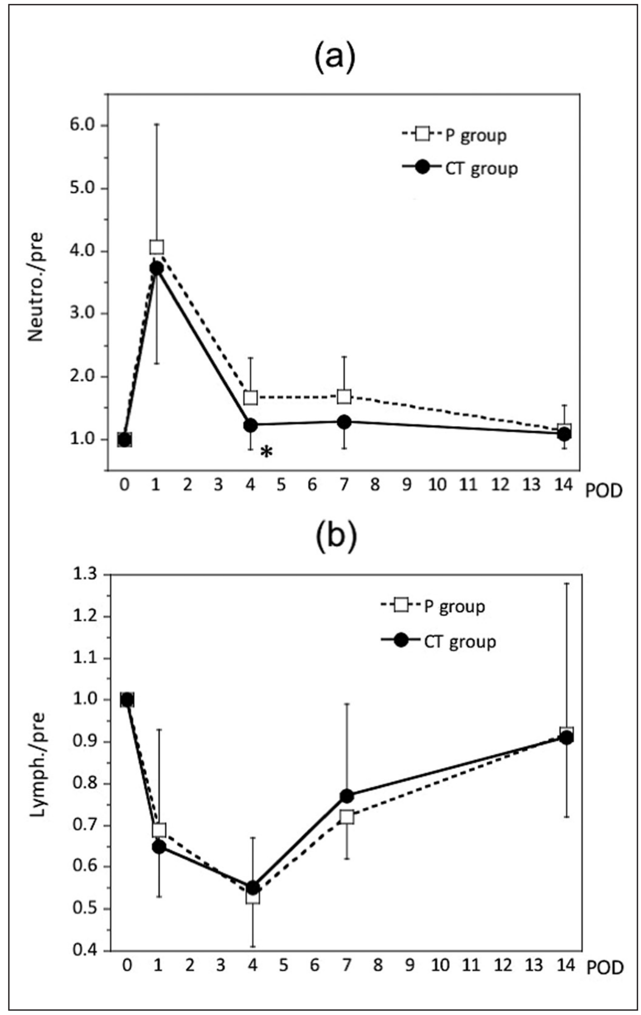

Figure 3. Changes in the ratios of neutrophil count (a) and lymphocyte count (b) relative to preoperative levels. All data are expressed as the mean ± SD. CT, cystine and theanine; POD, postoperative day. *$P < .05$ vs values in the placebo (P) group.

[relative to preoperative levels]), with 2 peaks observed on POD 1 and POD 5 (1569 ± 233 kcal, 1.11 ± 0.16). No increase was observed on POD 1 in the CT group (1396 ± 277 kcal, 0.99 ± 0.21). A single peak was observed on POD 5 (1504 ± 159 kcal, 1.06 ± 0.18), and this value decreased to preoperative levels on POD 7. REE (relative to preoperative levels) was significantly lower in the CT group than in the P group on POD 1 ($P = .043$) (Table 2).

In both groups, changes in body temperature from preoperative levels peaked on the day of surgery, but a faster decrease was observed in the CT group than in the P group. A significantly lower value ($P = .010$) was observed on POD 5 (Figure 5). The number of times that nonsteroidal anti-inflammatory drugs (NSAIDs) were used did not differ significantly (CT group, 1.80 ± 1.78 times; P group, 2.18 ± 3.13 times).

Discussion

The results of this study indicated that perioperative oral administration of 700 mg cystine and 280 mg theanine to gastric cancer patients treated by distal gastrectomy partly suppressed increases in REE after surgery and promoted rapid recovery of body temperature, IL-6 levels, CRP levels, neutrophil count, and total lymphocyte fraction.

In a small intestine manipulation model in mice that assumed gastrointestinal tract surgery, we also found that administering cystine and theanine for 4 days before surgery prevented GSH decreases in the intestinal tract and Peyer's patches, as well as lowered blood IL-6 concentrations.[21] Because GSH levels in the small intestine and Peyer's patches and IL-6 concentrations in blood were negatively correlated,

Table 2. REE (kcal/d) During the Perioperative Period

	Preoperative	POD 1	POD 4	POD 5	POD 7	POD 14
CT group	1414 ± 201 (1.00)	1396 ± 277 (0.99 ± 0.21)*	1402 ± 206 (0.99 ± 0.14)	1504 ± 159 (1.06 ± 0.18)	1418 ± 250 (1.00 ± 0.21)	1246 ± 193 (0.88 ± 0.17)
P group	1408 ± 241 (1.00)	1609 ± 330 (1.14 ± 0.16)	1566 ± 230 (1.11 ± 0.17)	1569 ± 233 (1.11 ± 0.16)	1498 ± 205 (1.06 ± 0.16)	1290 ± 160 (0.92 ± 0.16)

All data are expressed as means ± SD, with values in parentheses representing the ratio to preoperative levels. CT, cystine and theanine; P, placebo; POD, postoperative day; REE, resting energy expenditure.
*$P < .05$ vs the values in the P group.

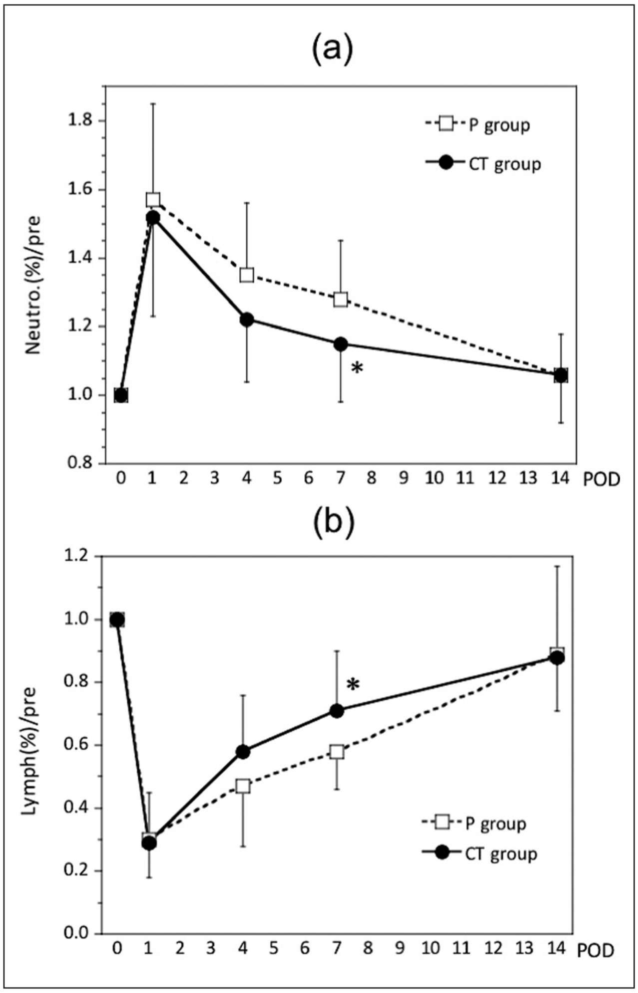

Figure 4. Changes in the ratios of neutrophil fraction (a) and lymphocyte fraction (b) relative to preoperative levels. All data are expressed as the mean ± SD. CT, cystine and theanine; POD, postoperative day, *$P < .05$ vs values in the placebo (P) group.

GSH increases in the small intestinal wall after oral cystine and theanine were thought to play a role in suppressing increased IL-6 levels.[21] In an in vitro study using the pulmonary epithelial cells of neonatal rats, lipopolysaccharide (LPS)–induced increases in IL-6 and tumor necrosis factor (TNF)–α were suppressed in response to increased GSH induced by addition of N-acetyl cysteine.[22] This response was cancelled by depleting

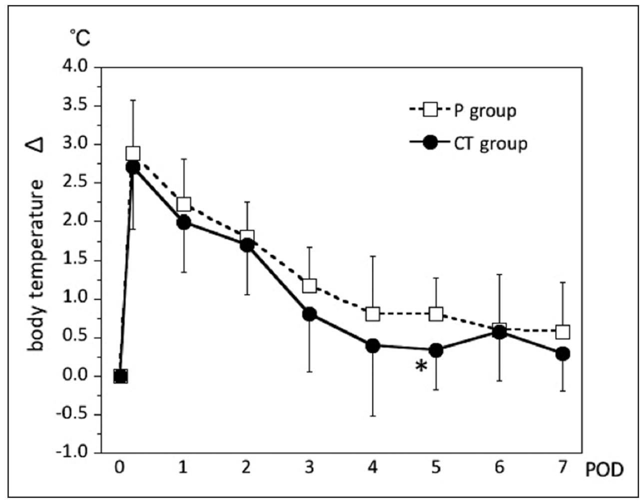

Figure 5. Changes in body temperature from preoperative levels over the perioperative period. All data are expressed as the mean ± SD. CT, cystine and theanine; POD, postoperative day. *$P < .05$ vs values in the placebo (P) group.

intracellular GSH through addition of a GSH synthesis inhibitor.[22] p38MAP kinase was shown to be related to this phenomenon.[22] In a clinical trial targeting surgery patients, cystine became essential during the perioperative period.[23] In addition, the synthetic pathway from methionine to cysteine remained inhibited in rats under surgical stress.[24] Considering these findings, suppressing GSH decreases in the small intestine with oral cystine and theanine may lead to reduced IL-6 production in response to abdominal surgery. However, further studies will be needed to elucidate the anti-inflammatory effects of cystine and theanine.

Further findings were obtained from a murine small intestine manipulation model. Locomotor activity, food intake, and weight in mice were significantly decreased after manipulation of the small intestine.[21] However, administering cystine and theanine for 4 days before surgery was associated with significantly faster recovery in these points than that of the control group.[21] Increased levels of inflammatory cytokines are known to induce various sickness behaviors, such as increased body temperature, reduced activity, and decreased appetite.[25,26] Rapid decreases in elevated IL-6 levels and body temperature were observed after distal gastrectomy in the present study.

These results suggest cystine and theanine may have the possibility of promoting recovery from sickness behaviors after abdominal surgery.

Five other studies on the effects of cystine and theanine administration in humans have been reported.[3,27-30] The first was a study of malnourished elderly patients in whom significantly increased antibody levels after influenza vaccinations were observed with administration of cystine and theanine.[27] The second was a report of significantly increased CRP levels, increased neutrophil counts, and decreased lymphocyte counts in athletes after strenuous exercise; excessive inflammation and immune suppression after exercise were reduced by administration of cystine and theanine.[3] The third was a study of runners participating in a long-distance relay race (*Eki-den*) where increases in granulocytes and decreases in lymphocytes after intense endurance exercise were reduced by cystine and theanine.[28] The fourth found a significantly reduced incidence of the common cold in adults who received cystine and theanine.[29] The fifth reported that continuous administration of cystine and theanine over 2 weeks of training suppressed reductions in natural killer (NK) cell activity in bodybuilders.[30] These results suggest that cystine and theanine can suppress excessive inflammation and increase the immune response after invasive processes, such as exercise or surgery.

IL-6 is a representative proinflammatory cytokine that is produced by macrophages and lymphocytes in response to invasion, and its level indicates the strength of a systemic inflammatory reaction. In addition, IL-6 itself promotes the production of acute-phase proteins in the liver, thereby acting as a mediator and promoting further inflammatory reactions.[31,32] It also acts on the hypothalamus-pituitary-adrenal axis; in recent years, IL-6 has been shown to induce immune suppression at a considerable level by stimulating secretion of glucocorticoids and catecholamines.[1,31-35]

CRP is an acute-phase protein that is produced by the liver in response to a stimulus by IL-6, and its levels reflect the strength of a systemic inflammatory reaction to surgical stress. In this study, both IL-6 and CRP levels recovered faster from their postsurgical increases in the CT group than in the P group, suggesting reduced systemic inflammation and reduced immune suppression.

During the perioperative period, neutrophils are released into the blood in response to the degree of inflammation, and increases in their numbers reflect a systemic inflammatory reaction to surgical stress.[36-40] In addition to IL-6 and CRP levels, neutrophil counts also showed early recovery in the present study, and oral administration of cystine and theanine appeared to reduce the inflammatory reaction.

In contrast to neutrophils, lymphocytes decrease with surgical stress.[40,41] In the present study, changes in total lymphocyte count did not differ significantly between the 2 groups. The blood lymphocyte fraction was significantly higher in the CT group on POD 7, but the effect of the lymphocyte fraction on the immune system during the perioperative period remains unclear. Lymphocytes play an important role in the immune system, but a detailed assessment of their function requires measuring both the cell count and cell types and activities. Therefore, further studies are necessary.

With regard to the daily dosage and duration of cystine and theanine treatment, the daily dosage used in the present study was the same as in other studies, but the duration of treatment varied from 10 to 35 days between studies.[3,27-30] In the present study, these amino acids were administered for 4 days prior to surgery, over a total of 10 days; the duration of this presurgical treatment was shorter than that in other studies. Extending the dosing period before and after the procedure may clarify its efficacy for reducing excessive inflammation and lymphocyte decreases; this issue should be examined in future studies.

REE has also been reported to increase according to the degree of operative invasion and the strength of inflammation.[33,42,43] The REE value 1 day after surgery was significantly lower in the CT group than in the P group, suggesting that the biological reaction against surgical stress was reduced in the CT group. Mochizuki et al[44] developed a burn injury model using guinea pigs and reported that early enteral nutrition (EN) suppressed increases in REE after injury, which emphasizes the importance of early EN during invasive procedures. Their report was the first to indicate that early EN suppresses increased REE after injuries. Other studies have reported suppression of increases in REE after gastrectomy managed with a fast-track surgery program for the first time.[45,46] Results of our study suggested that administration of cystine and theanine may also reduce REE increases after gastrointestinal surgery in humans, as described in the study with fast-track surgery.[45,46] There have been no previous reports on the relationship between these amino acids and REE after surgery.

Inflammation is a biological response that helps to maintain homeostasis, but excessive inflammation following excessive stress causes organ damage and compromised immune function; thus, it has a negative influence on short- and long-term prognoses.[1,34-36] Local inflammation due to surgery becomes systemic within 4–8 hours and induces systemic inflammatory response syndrome (SIRS), which results in increases in proinflammatory cytokine levels, a condition known as hypercytokinemia.[1,47] The effects of this excessive production of proinflammatory cytokines, such as IL-6, include propagation to other organs and organ damage (eg, tissue damage in the lungs due to neutrophil infiltration).[1,47-49] In addition, there have been several reports that reduced immune function, especially reduced cellular immunity during the perioperative period induced by the hypothalamus-pituitary-adrenal system, is related to metastatic relapses of malignant tumors.[1,50-52] Appropriate regulation of inflammatory reactions during the perioperative period is important in preventing the onset of organ damage and infectious complications, achieving a stable postoperative course and early recovery, and reducing the relapse rate from malignant tumors. From this perspective, an immunomodulating diet, including ω-3 fatty acids and arginine, that aims to reduce excessive inflammation during the perioperative period has been widely used in clinical settings recently.[2,53-56] We could not reveal the beneficial effects of cystine and theanine on the morbidity of postoperative complications in this study because no complications were observed after surgery. We also did not assess the length of hospital stay because hospital stay was designated to be longer than 14 postsurgical days by our clinical pathway. More detailed large-scale clinical studies using an intention-to-treat analysis with more invasive operative procedures that assess complication rates, lengths of hospital stays, and long-term prognoses should be conducted in the future. Also, more research is needed to determine if there is any interaction (synergistic or antagonistic) between common immune-modulating ingredients (including ω-3 fatty acid and arginine) and cystine/theanine. However, because it has been shown that oral administration of cystine and theanine tends to reduce inflammation after uneventful gastric cancer surgery, cystine and theanine may be used for immunonutrition.

Conclusions

This study suggested that oral administration of cystine and theanine during the perioperative period may alleviate post-gastrectomy inflammation and promote recovery after surgery. Future studies are expected to define the efficacy and mechanisms of cystine and theanine.

References

1. Kurosawa S, Kato M. Anesthetics, immune cells, and immune responses. *J Anesth*. 2008;22:263-277.
2. Sakurai Y, Masui T, Yoshida I, et al. Randomized clinical trial of the effects of perioperative use of immune-enhancing enteral formula on metabolic and immunological status in patients undergoing esophagectomy. *World J Surg*. 2007;31:2150-2157.
3. Murakami S, Kurihara S, Koikawa N, et al. Effects of oral supplementation with cystine and theanine on the immune function of athletes in endurance exercise: randomized, double-blind, placebo-controlled trial. *Biosci Biotechnol Biochem*. 2009;73:817-821.
4. Rimaniol AC, Mialocq P, Clayette P, Dormont D, Gras G. Role of glutamate transporters in the regulation of glutathione levels in human macrophages. *Am J Physiol Cell Physiol*. 2001;281:1964-1970.
5. Angelini G, Gardella S, Ardy M, et al. Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. *Proc Natl Acad Sci U S A*. 2002;99:1491-1496.
6. Asatoor AM. Tea as a source of urinary ethylamine. *Nature*. 1966;210:1358-1360.
7. Bukowski JF, Morita CT, Brenner MB. Human gamma delta T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity. *Immunity*. 1999;11:57-65.
8. Terashima T, Takido J, Yokogoshi H. Time-dependent changes of amino acids in the serum, liver, brain and urine of rats administered with theanine. *Biosci Biotechnol Biochem*. 1999;63:615-618.
9. Takagi Y, Kurihara S, Higashi N, et al. Combined administration of (L)-cystine and (L)-theanine enhances immune functions and protects against influenza virus infection in aged mice. *J Vet Med Sci*. 2010;72:157-165.
10. Sido B, Hack V, Hochlehnert A, Lipps H, Herfarth C, Dröge W. Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. *Gut*. 1998;42:485-492.
11. Fischman CM, Udey MC, Kurtz M, Wedner HJ. Inhibition of lectin-induced lymphocyte activation by 2-cyclohexene-1-one: decreased intracellular glutathione inhibits an early event in the activation sequence. *J Immunol*. 1981;127:2257-2262.
12. Hamilos DL, Zelarney P, Mascali JJ. Lymphocyte proliferation in glutathione-depleted lymphocytes: direct relationship between glutathione availability and the proliferative response. *Immunopharmacology*. 1989;18:223-235.
13. Ishii T, Sugita Y, Bannai S. Regulation of glutathione levels in mouse spleen lymphocytes by transport of cysteine. *J Cell Physiol*. 1987;133:330-336.
14. Satz GT, Bannister WH, Bannister JV. Free radicals and thiol compounds: the role of glutathione against free radical toxicity. In: Vina J, ed. *Glutathione: Metabolism and Physiological Functions*. Boca Raton, FL: CRC Press; 1990:237-257.
15. Kim JM, Kim H, Kwon SB, et al. Intracellular glutathione status regulates mouse bone marrow monocyte-derived macrophage differentiation and phagocytic activity. *Biochem Biophys Res Commun*. 2004;325:101-108.
16. Luo JL, Hammarqvist F, Andersson K, Wernerman J. Skeletal muscle glutathione after surgical trauma. *Ann Surg*. 1996;223:420-427.
17. Panel on Macronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Protein and amino acids. In: Food and Nutrition Board, Institute of Medicine of the National Academies, ed. *Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol*. Washington, DC: National Academies Press; 2005:711-712.
18. Panel of Health and Nutrition Promotion, Committee of Hygiene, Ministry of Health and Labor. *Nutritional Requirement of Japanese*. 6th ed [monograph on the Internet]. Tokyo, Japan: Ministry of Health and Labor; 1999. http://www1.mhlw.go.jp/shingi/s9906/s0628-1_11.html/
19. Kurihara S, Shibahara S, Arisaka H, Akiyama Y. Enhancement of antigen-specific immunoglobulin G production in mice by co-administration of L-cystine and L-theanine. *J Vet Med Sci*. 2007;69:1263-1270.
20. Wittekind C, Yamazaki S. Stomach. In: Sobin LH, Gospodarowicz MK, and Wittekind C, eds. *International Union Against Cancer (UICC) TNM Classification of Malignant Tumours*. 7th ed. New York: Wiley-Liss; 2010:73-77.
21. Shibakusa T, Mikami T, Kurihara S, et al. Enhancement of postoperative recovery by preoperative oral co-administration of the amino acids, cystine and theanine, in a mouse surgical model. *Clin Nutr*. 2012;31(4):555-561.
22. Haddad JJ. The involvement of L-gamma-glutamyl-L-cysteinyl-glycine (glutathione/GSH) in the mechanism of redox signaling mediating MAPK(p38)-dependent regulation of pro-inflammatory cytokine production. *Biochem Pharmacol*. 2002;63:305-320.
23. Dale G, Young G, Latner AL, Goode A, Tweedle D, Johnston ID. The effect of surgical operation on venous plasma free amino acids. *Surgery*. 1977;81:295-301.
24. Viña J, Gimenez A, Puertes IR, Gasco E, Viña JR. Impairment of cysteine synthesis from methionine in rats exposed to surgical stress. *Br J Nutr*. 1992;68:421-429.
25. Bluthé RM, Michaud B, Poli V, Dantzer R. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. *Physiol Behav*. 2000;70:367-373.
26. Dantzer R. Cytokine, sickness behavior, and depression. *Neurol Clin*. 2006;24:441-460.
27. Miyagawa K, Hayashi Y, Kurihara S, Maeda A. Co-administration of L-cystine and L-theanine enhances efficacy of influenza vaccination in elderly persons: nutritional status-dependent immunogenicity. *Geriatr Gerontol Int*. 2008;8:243-250.

28. Murakami S, Kurihara S, Titchenal CA, Ohtani M. Suppression of exercise-induced neutrophilia and lymphopenia in athletes by cystine/theanine intake: a randomized, double-blind, placebo-controlled trial. *J Int Soc Sports Nutr*. 2010;7:23.
29. Kurihara S, Hiraoka T, Akutsu H, Sukegawa E, Bannai M, Shibahara S. Effects of L-cystine and L-theanine supplementation on the common cold: a randomised, double-blind, and placebo-controlled trial. *J Amino Acids*. 2010;2010:307475.
30. Kawada S, Kobayashi K, Ohtani M, Fukusaki C. Cystine and theanine supplementation restores high-intensity resistance exercise-induced attenuation of natural killer cell activity in well-trained men. *J Strength Cond Res*. 2010;24:846-851.
31. Bethin KE, Vogt SK, Muglia LJ. Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. *Proc Natl Acad Sci U S A*. 2000;97:9317-9322.
32. Kotani G, Usami M, Kasahara H, Saitoh Y. The relationship of IL-6 to hormonal mediators, fuel utilization, and systemic hypermetabolism after surgical trauma. *Kobe J Med Sci*. 1996;42:187-205.
33. Haddad JJ, Saadé NE, Safieh-Garabedian B. Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis. *J Neuroimmunol*. 2002;133:1-19.
34. Greenfeld K, Avraham R, Benish M, et al. Immune suppression while awaiting surgery and following it: dissociations between plasma cytokine levels, their induced production, and NK cell cytotoxicity. *Brain Behav Immun*. 2007;21:503-513.
35. Ogawa K, Hirai M, Katsube T, et al. Suppression of cellular immunity by surgical stress. *Surgery*. 2000;127:329-336.
36. Matsuda T, Saito H, Fukatsu K, et al. Cytokine-modulated inhibition of neutrophil apoptosis at local site augments exudative neutrophil functions and reflects inflammatory response after surgery. *Surgery*. 2001;129:76-85.
37. Iwasaka H, Kitano T, Miyakawa H, et al. Neutrophilia and granulocyte colony-stimulating factor levels after cardiopulmonary bypass. *Can J Anaesth*. 2001;48:81-84.
38. Iwase M, Kondo G, Watanabe H, et al. Regulation of Fas-mediated apoptosis in neutrophils after surgery-induced acute inflammation. *J Surg Res*. 2006;134:114-123.
39. Petrovsky N, Harrison LC. Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? *J Immunol*. 1997;158:5163-5168.
40. Rem J, Brandt MR, Kehlet H. Prevention of postoperative lymphopenia and granulocytosis by epidural analgesia. *Lancet*. 1980;315:283-285.
41. Tayama E, Hayashida N, Oda T, et al. Recovery from lymphocytopenia following extracorporeal circulation: simple indicator to assess surgical stress. *Artif Organs*. 1999;23:736-740.
42. Luo K, Li JS, Li LT, Wang KH, Shun JM. Operative stress response and energy metabolism after laparoscopic cholecystectomy compared to open surgery. *World J Gastroenterol*. 2003;9:847-850.
43. Ross PJ, Lavelle EC, Mills KH, Boyd AP. Adenylate cyclase toxin from *Bordetella pertussis* synergizes with lipopolysaccharide to promote innate interleukin-10 production and enhances the induction of Th2 and regulatory T cells. *Infect Immun*. 2004;72:1568-1579.
44. Mochizuki H, Trocki O, Dominioni L, Brackett KA, Joffe SN, Alexander JW. Mechanism of prevention of postburn hypermetabolism and catabolism by early enteral feeding. *Ann Surg*. 1984;200:297-310.
45. Wang D, Kong Y, Zhong B, Zhou X, Zhou Y. Fast-track surgery improves postoperative recovery in patients with gastric cancer: a randomized comparison with conventional postoperative care. *J Gastrointest Surg*. 2010;14:620-627.
46. Kehlet H, Wilmore DW. Fast-track surgery. *Br J Surg*. 2005;92:3-4.
47. Raeburn CD, Sheppard F, Barsness KA, Arya J, Harken AH. Cytokines for surgeons. *Am J Surg*. 2002;183:268-273.
48. Thomas S, Karnik S, Balasubramanian KA. Surgical manipulation of the small intestine and its effect on the lung. *J Surg Res*. 2002;106:145-156.
49. Schwarz NT, Kalff JC, Türler A, et al. Selective jejunal manipulation causes postoperative pan-enteric inflammation and dysmotility. *Gastroenterology*. 2004;126:159-169.
50. Tartter PI, Steinberg B, Barron DM, Martinelli G. The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. *Arch Surg*. 1987;122:1264-1268.
51. Schantz SP, Brown BW, Lira E, Taylor DL, Beddingfield N. Evidence for the role of natural immunity in the control of metastatic spread of head and neck cancer. *Cancer Immunol Immunother*. 1987;25:141-148.
52. Fujisawa T, Yamaguchi Y. Autologous tumor killing activity as a prognostic factor in primary resected nonsmall cell carcinoma of the lung. *Cancer*. 1997;79:474-481.
53. Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nat Med*. 2003;9:517-524.
54. Akbarshahi H, Andersson B, Norden M, Andersson R. Perioperative nutrition in elective gastrointestinal surgery—potential for improvement? *Dig Surg*. 2008;25:165-174.
55. Jiang ZM, Wilmore DW, Wang XR, et al. Randomized clinical trial of intravenous soybean oil alone versus soybean oil plus fish oil emulsion after gastrointestinal cancer surgery. *Br J Surg*. 2010;97:804-809.
56. Okamoto Y, Okano K, Izuishi K, Usuki H, Wakabayashi H, Suzuki Y. Attenuation of the systemic inflammatory response and infectious complications after gastrectomy with preoperative oral arginine and omega-3 fatty acids supplemented immunonutrition. *World J Surg*. 2009;33:1815-1821.