EFFECT OF MODEST PERIOPERATIVE INTAKE OF IMMUNO-NUTRITION RICH IN ω-3 POLYUNSATURATED FATTY ACIDS ON IL-10 IN ESOPHAGEAL CANCER PATIENTS

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Abstract

Background Recent trials have shown that perioperative administration of an immuno-enhancing diet can reduce the incidence of infectious complications and shorten hospital stay following elective surgery. The purpose of this study was to evaluate whether a relatively small perioperative intake of ω-3 polyunsaturated fatty acid (PUFA)-rich nutritional supplement modulates postoperative inflammatory responses.

Methods Twenty-three patients with esophageal cancer were prospectively randomized into Treatment ($n=11$) and Control ($n=12$) groups. The former drank 2 packs (0.4 L) of a liquid diet that was supplemented with ω-3 fatty acids each day for 6 consecutive days prior to esophagectomy, while the latter drank a control liquid. In addition, both groups received postoperative enteral feeding with the same formula. The levels of human leukocyte antigen-DR (HLA-DR), interleukin (IL)-6 and IL-10 were measured, and polymorphonuclear cell and total lymphocyte counts were obtained.

Results Plasma IL-10 levels were significantly ($p<0.05$) higher in the Treatment group than the Control group after beginning supplemental nutrition. By contrast, postoperative IL-6 levels tended to lower in the Treatment group, but the difference did not reach the level of significance.

Conclusions Preoperative oral intake of a small amount of ω-3 PUFA-rich nutritional supplement increased postoperative IL-10 expression.

Key words: esophagectomy, esophageal cancer, immuno-nutrition, IL-10, ω-3 Polyunsaturated Fatty Acids

Introduction

The survival rate among esophageal cancer patients has improved in recent years due, in large part, to advances in surgical techniques and perioperative management[1,2]. Postoperative morbidity rates associated with esophagectomy also have declined, though they continue to be substantial[3,4]. To address this issue, greater attention is being paid to preventing surgery-related complications and to new strategies aimed at modulating postoperative inflammatory responses through administration of key nutrients. For instance, recent trials have shown that perioperative administration of an immuno-enhancing diet can significantly reduce the incidence of infectious complications[5-10] and shorten the patient's hospital stay[7,10] following elective surgery for gastrointestinal

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cancer.

Recent findings indicate that specific nutrients such as arginine, glutamine, ω-3 polyunsaturated fatty acids (PUFAs) and RNA may modulate immune and inflammatory responses, as well as gut function[5-11]. This suggests immuno-enhanced nutrition may produce clinical benefits for postoperative and critically ill patients. In particular, ω-3 PUFAs may exert effects that directly suppress immune function[12]. For instance, several studies have shown that ω-3 PUFAs reduce the production of a variety of inflammatory cytokines, including tumor necrosis factor (TNF)-α[13-17], interleukin (IL)-6[13-15], IL-1β[16] and IL-8[17], as well as arachidonic acid-derived eicosanoids such as prostaglandin E_2 (PGE_2)[16,18], thromboxane B2 (TXB2)[16] and leukotriene B4 (LTB4)[14,17]. Furthermore, enteral nutrition that includes ω-3 PUFAs reportedly leads to significantly greater expression of HLA-DR on monocytes in patients after severe trauma[19], and it was recently, reported that ω-3 PUFAs are associated with increased production of IL-10 in humans[20,21]. Thus ω-3 PUFAs appear to be potentially useful anti-inflammatory agents that preserve immune function better than standard formulas, while attenuating some aspects of the inflammatory response[22].

We were interested in the effects of perioperative enteral administration of ω-3 PUFAs to patients planning to undergo esophagectomy. We reasoned that esophageal cancer patients could benefit from immuno-enhanced nutrition, as they frequently suffer from malnutrition, endure greater surgical traumas and have higher complication rates than patients undergoing other surgical procedures. Moreover, in most previous studies the patients were asked to drink about 1 L/day of liquid diet prior to surgery[5-10], but this is not possible for many patients. In fact, the mean daily preoperative intake of the formula was only about 90% in one of those studies[9], and several percent of patients had to be excluded for lack of compliance in a number of the other studies[5,8,10]. We believed all of our patients would consider 0.4 L/day of liquid diet an acceptable nutritional supplement to their usual diet. If this small amount of oral immuno-enhancing nutrition had some beneficial effect for patients planning to undergo surgery, the cost would be reduced and use of immuno-enhancing nutrition as a preoperative preparation might be more prevalent. We therefore designed the present study to determine whether a relatively small amount of perioperative ω-3 PUFA-rich nutritional supplement would positively modulate postoperative inflammatory responses.

Methods

Patients and preoperative nutrition

Between December 2004 and December 2005, we conducted a randomized trial in the Department of Surgery, Akita University School of Medicine. The main inclusion criterion was operable thoracic esophageal cancer without severe dysphagia. Exclusion criteria included severe dysphagia that made the patient unable to take in adequate food, ages older than 75 years, preoperative chemoradiotherapy, liver cirrhosis and immunological diseases. All patients provided written informed consent after the details of the protocol were fully explained. The protocol was approved by the Ethical Committee of the Akita University School of Medicine.

Using sealed envelopes, patients were randomized to drink 2 packs of either a liquid diet supplemented with ω-3 PUFAs (ω-6/ω-3=3), [Racol (200 mL/pack), Otsuka Pharmaceutical, Tokyo, Japan] or a control liquid diet (ω-6/ω-3=44) [Ensure Liquid (250 mL/pack), Abbot, Tokyo, Japan] for 6 consecutive days (Table 1)[23]. Hospital admission was scheduled 6 days before surgery, and both groups of patients were asked to drink 2 packs of the liquid diet any time they liked each day until the evening of the day before surgery. During that period, they also ate the standard food provided by the hospital.

Surgery

All patients underwent esophagectomy with extended lymph node dissection through a right posterolateral thoracotomy and reconstruction with a gastric tube via the posterior mediastinal route. A tube jejunostomy was placed intraoperatively using the modified Witzel technique for postoperative enteral feeding.

Postoperative nutrition

After surgery, all patients received enteral feeding with the same liquid diet taken preoperatively and were on the

Table 1. Comparison of the supplemental diets (per 100 ml)[23]

Component	Treatment group Racol	Control group Ensure
Energy (kcal) (protein/fat/carbohydrate)	100 (18/20/62)	100 (14.1/31.5/54.5)
Protein		
Casein protein (g)	3.4	3.4
Soy protein (g)	1.7	0.5
Fat		
Medium-chain fatty acid (mg)	716	3.5
Myristic acid (mg)	0	14.1
Palmic acid (mg)	269	415.4
Stearic acid (mg)	72	84.5
Oleic acid (mg)	342	929.3
Linolenic acid (mg)	450	1,992.3
α-Linolenic acid (mg)	150	45.8
Arachidonic acid (mg)	0	21.1
ω-6 FA (mg)	450	2,013.4
ω-3 FA (mg)	150	45.8
ω-6/ω-3	3	44
Carbohydrate (g)	15.6	13.7
Vitamins and trace elements		
Vitamin A (IU)	207	250
Vitamin E (IU)	0.7	3
Vitamin C (mg)	28.1	15.2
Folate (μg)	37.5	20
Vitamin B_6 (mg)	0.5	0.2
Vitamin B_{12} (μg)	0.6	0.6
Iron (mg)	0.6	0.9
Copper (mg)	0.1	0.1
Manganese (mg)	0.1	0.2
Zinc (mg)	0.6	1.5

FA, fatty acid ; Vit, vitamin

same schedule. Enteral nutrition was started at 200 mL/day on postoperative day 3, after which the volume was progressively increased to a maximum of 1,000 mL/day by postoperative day 7. Beginning on postoperative day 1, all patients also received continuous infusion of parenteral nutrition (1,120 kcal/day) through a central venous catheter. On postoperative day 8, the anastomosis was checked for leakage using a water-soluble contrast medium. If no leakage was observed, the patient was started on an oral diet on postoperative day 9, and the volume of enteral nutrition was reduced to 800 mL/day on postoperative day 11. That volume of enteral nutrition was then continued until the day of discharge from the hospital (Fig. 1).

Blood sample

Peripheral blood was collected from all patients before starting the oral intake of liquid diet (day −6), 1 day before surgery (day −1), 1 day after surgery (day +1), 2 days after surgery (day +2), 7 days after surgery (day +7) and 14 days after surgery (day +14). The collected samples were used to obtain polymorphonuclear cell (PMN) counts, total lymphocyte counts and plasma levels of C reactive protein (CRP). Additional blood samples

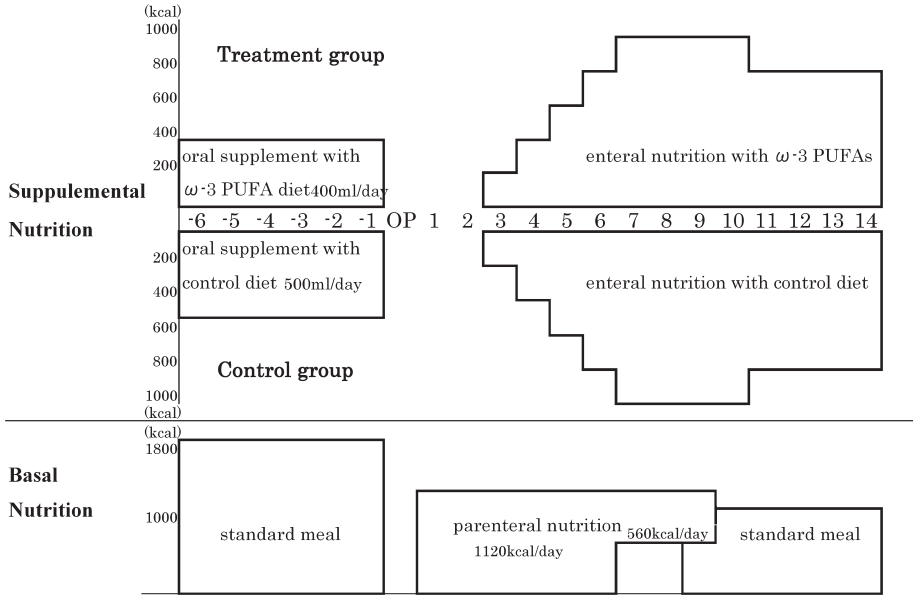

Fig. 1. Perioperative nutritional plan ; OP, the day of operation

were used to measure the plasma levels of IL-6 and IL-10 using specific enzyme-linked immunosorbent assays, and to assess expression of human leukocyte antigen-DR (HLA-DR) on CD14+ monocytes. For monocyte CD14/HLA-DR staining and flow cytometric analysis, 100 μl of whole blood collected into sodium citrate-containing tubes were incubated for 15 min in the dark at room temperature with 10 μl of fluorescein isothiocyanate (FITC)-conjugated anti-CD14 (DAKO A/S, Glostrup, Denmark) and 20 μl of phycoerythrin (PE)-conjugated anti-HLA-DR (Becton Dickinson, San Jose, CA). In addition, 100 μl of the same sample were stained with 10 μl of FITC-conjugated anti-CD14 (Dako) and 20 μl of PE-conjugated mouse IgG2a (Becton Dickinson) as an isotype control. After staining, 2 ml of ammonium chloride solution were added to each tube, and the samples were incubated for 15 min in the dark at room temperature and then washed twice in PBS. The cells were then input to a flow cytometer (FACSCalibur ; Becton Dickinson) and analyzed using Cell Quest software (Becton Dickinson). We evaluated the Geometric mean (Geo mean) ratio (Geo mean of the HLA-DR fluorescence intensity/Geo mean of the isotype control fluorescence intensity) as an index of the number of HLA-DR molecules expressed on CD14-positive cells.

Statistics analysis

In order to standardize the response profiles and make the results more comparable, parameters were expressed as percentages of the baseline (day −6) values (Fig. 2, 3B, 4B). The baseline value was set as 100%. There were no differences in the absolute baseline values of the described parameters between the two groups (data not shown). The data are shown as means±SD. The non-parametric Mann-Whitney U test and Student's t test were used to compare continuous variables, and the χ^2 test was used to compare discrete variables (baseline characteristics and clinical outcomes). Variation in the time course of the Geo mean ratio for HLA-DR, cytokines and PMN counts were compared using repeated measures ANOVA. Values of $p<0.05$ were considered significant.

Results

Table 2 summarizes the characteristics of the Treatment and Control groups. There were no significant differences with respect to age, sex, preoperative total pro-

Table 2. Comparison of the clinical and surgical characteristics

	Treatment group (n=11)	Control group (n=12)	p Valu
Age	69±4.8	66±5.4	N.S.
Male/Female	10/1	11/1	N.S.
Body mass index	21.2±2.2	21.2±2.8	N.S.
Preoperative Total protein	6.6±0.4	6.5±0.5	N.S.
Preoperative Albumin	4.0±0.2	4.0±0.4	N.S.
Number of patients with body weight loss of more than 2 kg after disease	2	2	N.S.
Operating time (min)	546±110	515±98	N.S.
Operaitve blood loss (ml)	668±331	666±389	N.S.
pStage I	3	2	N.S.
II	3	5	
III	4	4	
IVa	1	1	

pStage, pathological classification based on the Guideline for Clinical and Pathologic Studies on Carcinoma of Esophagus, Japanese Society for Esophageal Diseases[24,25].

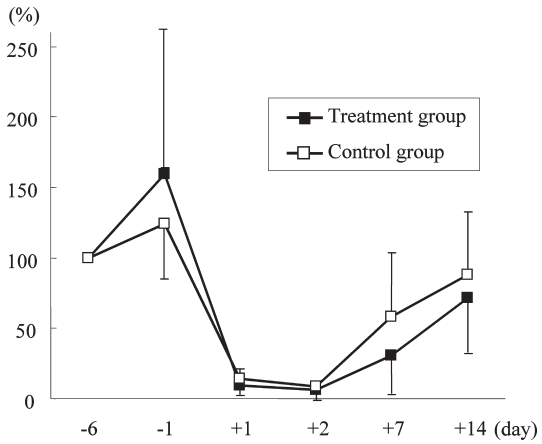

Fig. 2. HLA-DR
Percentages of the baseline (day −6) values of Geo mean ratios, which served as an index of the number of HLA-DR molecules expressed on CD14-positive cells in the Treatment and Control groups

tein, albumin, preoperative body mass index (BMI), operating time, operative blood loss or pathological stage[24,25] between the two groups. All patients completed preoperative oral intake of 0.4-0.5 L (2 packs) of liquid diet each day and the postoperative enteral nutrition through a tube-jejunostomy.

On day −1 (after being on the liquid diet for 5 days) the Geo mean ratio for HLA-DR was higher in the Treatment group than the Control group, but the difference was not significant (Fig. 2). Following surgery, levels declined in both groups.

Plasma IL-10 levels were significantly higher in the Treatment group than the Control group (p=0.016) (Fig. 3). By contrast, plasma IL-6 levels were lower in the Treatment group than the Control group at all sample times, though the difference was not significant (p=0.31) (Fig. 4).

There was no difference in the PMN counts between the two groups at any sample time (Fig. 5). There were also no significant differences in the total lymphocyte counts or plasma CRP levels when expressed as percentages of the baseline (day −6) values (data not shown).

There were no complications related to enteral nutrition, and no significant differences between the two groups with respect to outcome variables, anastomotic leak, other complications or the length of hospital stay (excluding patients who underwent subsequent postoperative adjuvant chemotherapy ; Table 3).

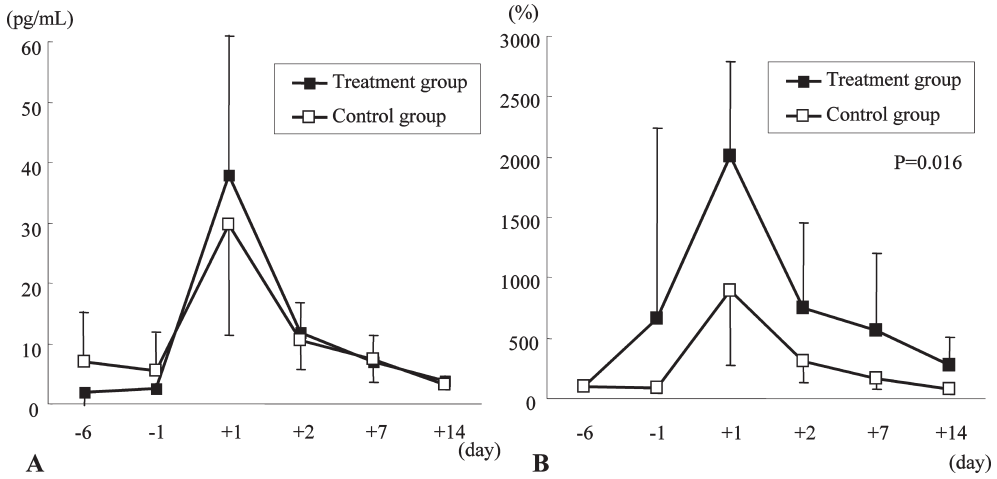

Fig. 3. IL-10
A : Absolute values of mean plasma IL-10 levels.
B : Percentages of the baseline (day –6) values of mean plasma IL-10 levels ($p=0.016$)

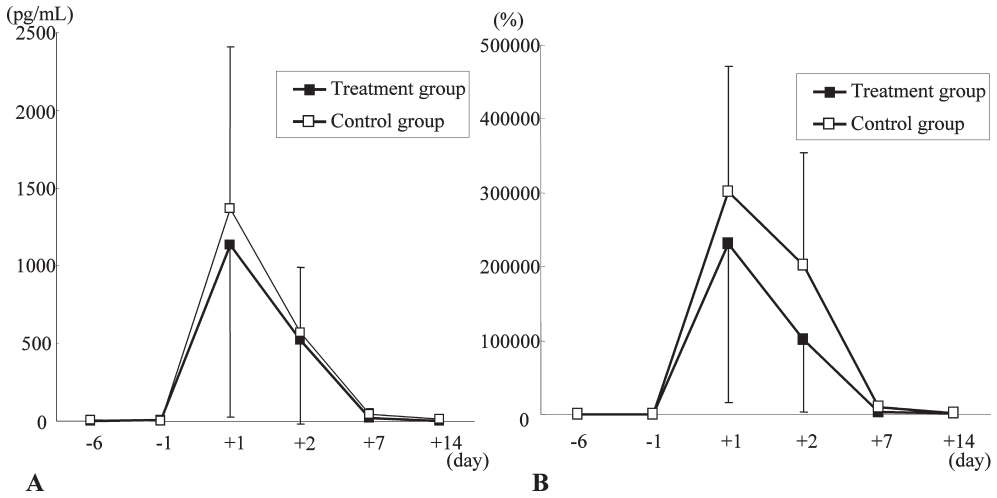

Fig. 4. IL-6
A : Absolute values of mean plasma IL-6 levels.
B : Percentages of the baseline (day –6) values of mean plasma IL-6 levels

Discussion

In our study, preoperative intake of a small amount of ω-3 PUFA-rich supplement increased plasma IL-10 levels in esophageal cancer patients. To the best of our knowledge, there have been no studies evaluating the effect of perioperative ω-3 PUFA-rich supplement on postoperative IL-10 levels.

IL-10 is an important anti-inflammatory cytokine expressed during immune responses in humans[26]. It is a potent deactivator of monocyte/macrophage proinflammatory cytokine synthesis[27] and is thought to be released to avoid excessive production of proinflammatory cytokines, which can lead to multiple organ failure[28]. Human

Table 3. Comparison of the clinical outcomes

	Treatment group (n=11)	Control group (*n*=12)	*p* Valu
Anastomotic leak	3	2	N.S.
Other complications	1	3	N.S.
pneumonia	0	1	
plumonary embolism	0	1	
chylothoracs	0	1	
acute cardiac infarction	1	0	
Hospital death	0	0	N.S.
Hospital stay	25±6.3	25.6±7.0	N.S.

Length of hospital stay is expressed as the mean±SD, excluding 3 patients in each groups who underwent sequential adjuvant chemotherapy.

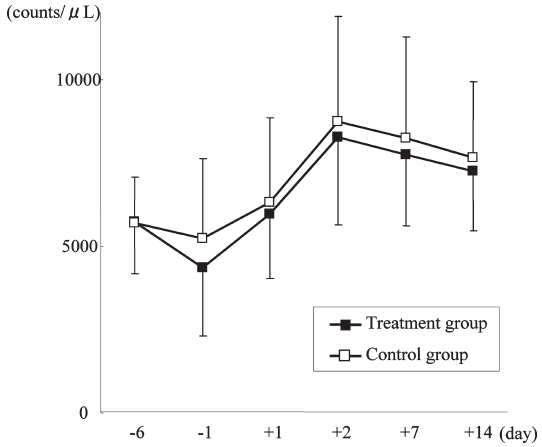

Fig. 5. PMN
Mean PMN counts.

volunteers given recombinant IL-10 after an endotoxin challenge suffer fewer systemic symptoms and neutrophil responses, and show less cytokine production than placebo-treated control subjects[29]. In addition, administration of recombinant IL-10 to experimental animal models of endotoxemia improves survival[27]. On the other hand, high levels of IL-10 and reduced levels of TNF-α are reportedly associated with a fatal outcome in febrile patients with community-acquired infection[30]. Thus IL-10 generally protects the host from systemic inflammation after toxin-induced injury, but can render the host susceptible to potentially lethal infection[26].

Preoperative administration of corticosteroids to esophageal cancer patients induces IL-10 expression and suppresses the release of proinflammatory cytokines, thereby reducing postoperative morbidity and attenuating postoperative inflammatory responses[31]. Similarly, in our study preoperative administration of ω-3 PUFA-rich supplement to esophageal cancer patients increased postoperative IL-10 expression.

Omega-3 PUFA competes with arachidonic acid, a member of the ω-6PUFA family, during prostaglandin and leukotriene synthesis. This suggests the attenuated inflammatory response seen after ingestion of ω-3 PUFAs is likely a direct effect of replacing arachidonic acid as an eicosanoid substrate and an indirect effect of altering the expression of inflammatory genes through effects on transcription factor activation[19,24]. Although the mechanism by which ω-3 PUFA induces IL-10 expression remains unknown, the relationship between ω-3 PUFAs and IL-10 in humans was recently described. The production of IL-10 in ConA-stimulated lymphocytes was significantly increased after two months of supplementation with ω-3 PUFAs in healthy human volunteers[20]. In addition, an epidemiological study that included 1123 participants in Chianti, Italy found that lower plasma levels of docosahexaenoic acid (DHA), a ω-3 PUFA family member, were strongly associated with lower IL-10 levels, and that the ω-6/ ω-3 ratio is a strong negative correlate of serum IL-10 levels[21]. IL-10 thus appears to be a key mediator of the anti-inflammatory effects of ω-3 PUFA.

We also detected increased preoperative HLA-DR ex-

pression and reduced postoperative plasma IL-6 levels, but these changes did not reach the level of significance. Furthermore perioperative ω-3 PUFA supplementation was not associated with a significant clinical benefit, such as a lower incidence of complications or a shorter hospital stay. This may be because our study sample was too small to have sufficient power to show significant clinical benefits. Alternatively, it may be that the amount of preoperative ω-3 PUFA supplement taken by the patients was not enough to elicit postoperative clinical benefits, or that the enteral feeding formula, which was enriched with ω-3 PUFAs, but not RNA or arginine, was not sufficient to elicit clinical benefits. Most of the earlier studies that did show clinical benefits evaluated the effect of supplemental liquid diets that were enriched with ω-3 PUFA, arginine and RNA (Oral Impact), and the patients took 1 L/day for 5-10 days preoperatively[4-10]. Only one study evaluated the anti-inflammatory effects of ω-3 PUFA using the same formula that we used (Racol)[23].

The optimal daily intake of preoperative immune-enhancing nutrition and the duration of administration needed to achieve the most beneficial postoperative effects are unknown. There has been no study in which the smallest effective level of preoperative immune-enhancing nutrition was determined. Of course, it may be that the larger the daily intake and the longer the duration of administration, the greater the benefit, but it would be desirable to know the daily intake and duration that was the most cost-effective and acceptable for all patients. In considering how a small daily intake of ω-3 PUFAs increased relative plasma IL-10 levels in the present study, it is important to remember that a large amount of ω-6 PUFA was included in the control diet (10 g/day), and this daily intake of ω-6 PUFAs could have enhanced the anti-inflammatory effect of ω-3 PUFAs. Moreover, because ω-3 PUFAs and ω-6 PUFAs use the same series of enzymes in their metabolic pathway, they compete with one another[32], which may have diminished the effect of ω-3 PUFAs in the Control group. It is also noteworthy that esophagectomy with extended lymph node dissection is a highly stressful surgical procedure and, therefore, one of the most suitable for evaluating inflammatory responses[31]. Although, this study had only one significant result in which plasma IL-10 levels were increased, it suggests the possibility that a relatively small amount of preoperative ω-3 PUFA-rich supplemental nutrition is effective. A larger study will be required to test that idea, however.

Conclusion

We have shown that a relatively small preoperative oral intake and postoperative enteral intake of a ω-3 PUFA-rich nutritional supplement induces postoperative IL-10 expression, which could have postoperatively beneficial anti-inflammatory effects. Although, this was a small preliminary study, our findings suggest the possibility that ω-3 PUFA-rich nutritional supplement has a beneficial effect when given preoperatively to patients who will undergo esophagectomy.

References

1) Ando, N., Ozawa, S., Kitagawa, Y., Shinozawa, Y. and Kitajima, M. (2000) Improvement in the results of surgical treatment of advanced squamous esophageal carcinoma during 15 consecutive years. *Ann. Surg.*, **232**, 225-232.

2) Abo, S., Kitamura, M., Hashimoto, M., Izumi, K., Minamiya, Y. and Shikama, T. (1996) Analysis of results of surgery performed over a 20-year period on 500 patients with cancer of the thoracic esophagus. *Surg. Today*, **26**, 77-82.

3) Atkins, B.Z., Shah, A.S., Hutcheson, K.A., Mangum, J.H., Pappas, T.N., Harpole, D.H. and Jr, D'Amico, T.A. (2004) Reducing hospital morbidity and mortality following esophagectomy. *Ann. Thorac. Surg.*, **78**, 1170-1176.

4) Ueda, Y., Osada, H. and Osugi, H. (2007) Thoracic and cardiovascular surgery in Japan during 2005. Annual report by the Japanese Association for Thoracic Surgery. *Gen. Thorac. Cardiovasc. Surg.*, **55**, 377-399.

5) Senkal, M., Zumtobel, V., Bauer, K.H., Marpe, B., Wolfram, G., Frei, A., Eickhoff, U. and Kemen, M. (1999) Outcome and cost-effectiveness of perioperative enteral immunonutrition in patients undergoing elective upper gastrointestinal tract surgery : a prospective randomized study. *Arch. Surg.*, **134**, 1309-

1316.

6) Gianotti, L., Braga, M., Fortis, C., Soldini, L., Vignali, A., Colombo, S., Radaelli, G. and Di, Carlo, V. (1999) A prospective, randomized clinical trial on perioperative feeding with an arginine-, omega-3 fatty acid-, and RNA-enriched enteral diet : effect on host response and nutritional status. *J. Parenter. Enteral. Nutr.*, **23**, 314-320.

7) Braga, M., Gianotti, L., Radaelli, G., Vignali, A., Mari, G., Gentilini, O. and Di, Carlo, V. (1999) Perioperative immunonutrition in patients undergoing cancer surgery : results of a randomized double-blind phase 3 trial. *Arch. Surg.*, **134**, 428-433.

8) Tepaske, R., Velthuis, H., Oudemans-van, Straaten, H.M., Heisterkamp, S.H., van Deventer, S.J., Ince, C., Eÿsman, L. and Kesecioglu, J. (2001) Effect of preoperative oral immune-enhancing nutritional supplement on patients at high risk of infection after cardiac surgery : a randomised placebo-controlled trial. *Lancet*, **358**, 696-701.

9) Braga, M., Gianotti, L., Vignali, A. and Carlo, V.D. (2002) Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery*, **132**, 805-814.

10) Braga, M., Gianotti, L., Nespoli, L., Radaelli, G. and Di, Carlo, V. (2002) Nutritional approach in malnourished surgical patients : a prospective randomized study. *Arch. Surg.*, **137**, 174-180.

11) Matsuda, A., Furukawa, K., Takasaki, H., Suzuki, H., Kan, H., Tsuruta, H., Shinji, S. and Tajiri, T. (2006) Preoperative oral immune-enhancing nutritional supplementation corrects TH1/TH2 imbalance in patients undergoing elective surgery for colorectal cancer. *Dis. Colon Rectum*, **49**, 507-516.

12) Grimble, R.F. (2001) Nutritional modulation of immune function. *Proc. Nutr. Soc.*, **60**, 389-397.

13) Trebble, T., Arden, N.K., Stroud, M.A., Wootton, S.A., Burdge, G.C., Miles, E.A., Ballinger, A.B., Thompson, R.L. and Calder, P.C. (2003) Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *Br. J. Nutr.*, **90,** 405-412.

14) Wachtler, P., Konig, W., Senkal, M., Kemen, M. and Koller, M. (1997) Influence of a total parenteral nutrition enriched with omega-3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. *J. Trauma.*, **42**, 191-198.

15) Wallace, FA., Miles, E.A. and Calder, P.C. (2003) Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. *Br. J. Nutr.*, **89**, 679-689.

16) Caughey, G.E., Mantzioris, E., Gibson, R.A., Cleland, L.G. and James, M.J. (1996) The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *Am. J. Clin. Nutr.*, **63**, 116-122.

17) Pacht, E.R., DeMichele, S.J., Nelson, J.L., Hart, J., Wennberg, A.K. and Gadek, J.E. (2003) Enteral nutrition with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants reduces alveolar inflammatory mediators and protein influx in patients with acute respiratory distress syndrome. *Crit. Care Med.*, **31**, 491-500.

18) Endres, S., Ghorbani, R., Kelley, V.E. *et al.* (1989) The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.*, **320**, 265-271.

19) Weimann, A., Bastian, L., Bischoff, W.E., Grotz, M., Hansel, M., Lotz, J., Trautwein, C., Tusch, G., Schlitt, H.J. and Regel, G. (1998) Influence of arginine, omega-3 fatty acids and nucleotide-supplemented enteral support on systemic inflammatory response syndrome and multiple organ failure in patients after severe trauma. *Nutrition*, **14**, 165-172.

20) Calder, P.C. (2004) n-3 fatty acids, inflammation, and immunity—relevance to postsurgical and critically ill patients. *Lipids*, **39**, 1147-1161.

21) Gorjão, R., Verlengia, R., Lima, T.M. *et al.* (2006) Effect of docosahexaenoic acid-rich fish oil supplementation on human leukocyte function. *Clin. Nutr.*, **25**, 923-938.

22) Ferrucci, L., Cherubini, A., Bandinelli, S., Bartali, B., Corsi, A., Lauretani, F., Martin, A., Andres-Lacueva, C., Senin, U. and Guralnik, J.M. (2006) Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J. Clin. Endocrinol. Metab.*, **91**, 439-446.

23) Aiko, S., Yoshizumi, Y., Tsuwano, S., Shimanouchi,

M., Sugiura, Y. and Maehara, T. (2005) The effects of immediate enteral feeding with a formula containing high levels of omega-3 fatty acids in patients after surgery for esophageal cancer. *J. Parenter. Enteral. Nutr.*, **29**, 141-147.

24) Japanese Society for Esophageal Diseases. (2004) Guidelines for clinical and pathologic studies on carcinoma of the esophagus, ninth edition : part I. *Esophagus*, **1**, 61-88.

25) Japanese Society for Esophageal Diseases. (2004) Guidelines for clinical and pathologic studies on carcinoma of the esophagus, ninth edition : part II. *Esophagus*, **1**, 107-125.

26) Opal, S.M. and DePalo, V.A. (2000) Anti-inflammatory cytokines. *Chest*, **117**, 1162-1172.

27) Howard, M., Muchamuel, T., Andrade, S. and Menon, S. (1993) Interleukin 10 protects mice from lethal endotoxemia. *J. Exp. Med.*, **177**, 1205-1208.

28) Hasselmann, M. and Reimund, J.M. (2004) Lipids in the nutritional support of the critically ill patients. *Curr. Opin. Crit. Care*, **10**, 449-455.

29) Pajkrt, D., Camoglio, L., Tiel-van, Buul, M.C., de Bruin, K., Cutler, D.L., Affrime, M.B., Rikken, G., van, der Poll, T., ten Cate, J.W. and van Deventer, S.J. (1997) Attenuation of proinflammatory response by recombinant human IL-10 in human endotoxemia : effect of timing of recombinant human IL-10 administration. *J. Immunol.*, **158**, 3971-3977.

30) Van, Dissel, J.T., van, Langevelde, P., Westendorp, R.G., Kwappenberg, K. and Frolich, M. (1998) Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet*, **351**, 950-953.

31) Sato, N., Koeda, K., Ikeda, K., Kimura, Y., Aoki, K., Iwaya, T., Akiyama, Y., Ishida, K., Saito, K. and Endo, S. (2002) Randomized study of the benefits of preoperative corticosteroid administration on the postoperative morbidity and cytokine response in patients undergoing surgery for esophageal cancer. *Ann. Surg.*, **236**, 184-190.

32) Calder, P.C. (2005) Polyunsaturated fatty acids and inflammation. *Biochem. Soc. Trans.*, **33**, 423-427.