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ORIGINAL COMMUNICATION

Effect of c-reactive protein and interleukins blood levels in postsurgery arginine-enhanced enteral nutrition in head and neck cancer patients

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Objective: It is known that the immune system is frequently affected in patients with head and neck cancer. Although immune dysfunction could be multifactorial, this immune system may be modulated by specific nutritional substrates, such as arginine. The aim of our study was to evaluate the effect of enteral nutrition supplemented with arginine on c-reactive protein (CRP), interleukin 6 (IL-6) and tumour necrosis factor (TNFα) in surgical head and neck cancer patients.
Design: Randomized trial.
Setting: Tertiary care.
Subjects: A population of 36 patients with oral and laryngeal cancer were enrolled.
Interventions: At surgery patients were randomly allocated to two groups: (a) patients receiving an enteral diet supplements with arginine and dietary fibre (group I, $n=18$); (b) patients receiving an isocaloric, isonitrogenous enteral formula (group II, $n=18$). Perioperatively and on postoperative day 5 the following parameters were evaluated: serum values of prealbumin, transferrin, albumin, total number of lymphocytes, interleukin 6, tumour necrosis factor α and c-reactive protein.
Results: The mean age was 59.6 ± 10.9 y (two females/34 males). No significant intergroup differences in the trend of the three plasma proteins and weight were detected. CRP decreased in both groups (group I: 152.9 ± 76.9 *vs* 68.9 ± 82.5 mg/dl; $P<0.05$; and group II: 105.9 ± 92 *vs* 43.6 ± 59.1 mg/dl; $P<0.05$). Interleukin 6 did not change (group I: 16.3 ± 12.3 *vs* 35.6 ± 83.4 pg/ml; NS; and group II: 22.8 ± 40 *vs* 9.9 ± 17.7 pg/ml; NS). TNFα did not show any differences (group I: 4.6 ± 1.6 *vs* 5.1 ± 1.5 pg/ml; NS; and group II: 8.8 ± 6.1 *vs* 5.8 ± 1.7 pg/ml; NS). Lymphocytes increased in both groups (group I: 1405.6 ± 517 *vs* $1634 \pm 529 \times 10^6$/ml; $P<0.05$; and group II: 1355 ± 696 *vs* $1561 \pm 541 \times 10^6$/ml; $P<0.05$).
Conclusions: Enhanced formula did not change IL6 and TNFα levels. Further studies are needed to determine whether route of nutrition or type of formula is the key in these patients.
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Keywords: head and neck cancer; interleukin 6; surgery; tumour necrosis factor α

Introduction

It is known that the immune system is frequently affected in patients with head and neck cancer. Surgery and malnutrition (which is frequent in these patients) have also been found to depress the immune system (Van Bokhorst-de van der Schueren *et al*, 1998). The alterations in the host defence mechanism make patients susceptible to the above-mentioned complications. Although immune dysfunction is multifactorial, the immune system may be modulated by specific nutritional substrates, such as arginine (Daly *et al*, 1988). Supplementation with arginine improved wound healing and enhanced immune function in animals by decreasing the T cell dysfunction associated with injury (Barbul *et al*, 1990). Other studies have shown that arginine increased lymphocytic interleukin-2 production and augmented lysis of tumours by macrophages (Barbul *et al*,

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1990). There is evidence suggesting that enteral nutrition, supplemented with different agents including arginine and dietary fibre, improves immune function and reduces postoperative complications, in different groups of patients such as pancreatic surgery (Di Carlo *et al*, 1999), surgery of stomach and colo-rectum cancer (Gianoti *et al*, 1999; Wu *et al*, 2001), critically ill patients (Caparros *et al*, 2001) and head and neck cancer patients (Van Bokhorst-de van der Schueren *et al*, 2001).

The aim of our study was to evaluate the effect of enteral nutrition supplemented with arginine on c-reactive protein (CRP), interleukin 6 (IL-6) and tumour necrosis factor (TNFα) in surgical head and neck cancer patients.

Material and methods

Patients

A population of 36 patients with a previous weight loss of 5–10% (6 months) and oral or laryngeal cancer was enrolled. Exclusion criteria included severely impaired hepatic functon (total bilirubin concentration >3.5 mg/dl) and renal function (serum creatinine concentration >2.5 mg/dl), ongoing infections, autoimmune disorders, steroids treatment and well-nourished (weight loss <10% of body weight). The study was a prospective concealed randomized trial carried out from March 1999 to January 2001. The study was blinded (patients and investigator). Baseline studies on all patients consisted of complete history taking and physical examination. General assessment of nutritional status included measurements of height, body weight, body mass index (BMI; kg/m^2). Informed consent was provided by patients.

Nutrition

At surgery patients were randomly allocated (sealed envelopes) to two groups: (a) patients receiving an enteral diet supplements with arginine (group I, $n=18$); (b) patients receiving an isoenergetic, isonitrogenous enteral formula (group II, $n=18$). Table 1 shows the composition of the two enteral diets. Enteral feeding was started within 24 h of surgery at a rate of 20 ml/h, via an intraoperatively placed nasogastric tube. The infusion rate was progressively increased every 24 h until the daily nutritional goal (32 total kcal/kg; 1.7 g protein/kg) was reached, on postoperative day 4. All patients reached 100% of calculated requirements. Group I received a daily dose of argine of 12.5 g during an average of 20 days. Prophylactic antibiotic treatment was given for 3 days postoperatively (ceftazidime, 500 mg three times daily i.v. and clyndamicine 300 mg three times daily i.v.).

Table 1 Composition of enteral diet (per 100 ml)

	Group I (immunonutrition)	*Group II (standard)*
Total energy (kcal)	125	125
Protein (g)	6.22	6.25
Free L-arginine	0.625	—
Casein	5.595	6.25
Total lipid (g)	4.86	4.86
W6/w3	5/1	5/1
Linoleic acid	1.18	1.25
α-Linolenic acid	0.23	0.25
Carbohydrate (g)	13.58	14.11
Dietary fibre (g)	0.9	—

Dietary fibre: oligofructose, inulin, soy polysaccharide, resistant starch, arabic gum, cellulose.

Patient monitoring

Perioperatively and on postoperative day 5 the following parameters were evaluated: serum values of prealbumin (mg/dl), transferrin (mg/dl), albumin (g/dl), total number of lymphocytes (10^6/ml), IL-6 (pg/ml), a TNFα (pg/ml) and CRP (mg/dl).

Assays

Fasting blood samples were drawn for measurement of albumin (3.5–4.5 g/dl), prealbumin (18–28 mg/dl), transferrin (250–350 mg/dl), and lymphocytes ($1.2-3.5\times10^6$/ml) with an autoanalyser (Hitachi, ATM, Mannheim, Germany).

Interleukins were measured by immulite analyser (DPC, Los Angeles, CA, USA). A 100 μl aliquot of serum (heparinized plasma) was required to determine IL-6 and TNFα; analytical sensivity of IL-6 and TNFα was 5 and 1.7 pg/ml, respectively. CRP was measured by immunoturbimetry (Roche Diagnostcis GmbH, Mannheim, Germany); analytical sensivity 0.5 mg/dl. Samples were assayed in duplicate in one day by the same investigator to avoid inter-investigator variability.

Statistical analysis

The results were expressed as average ± standard deviation. The distribution of variables was analysed using the Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analysed with two-tailed paired or unpaired Student's *t*-test, as needed and analysis of variance (ANOVA). Non-parametric variables were analysed with the Friedman–Wilcoxon tests. To minimize the potential for introducing bias, all randomized patients were included in the comparisons, irrespective of whether or not and for how long they complied with their allocated regimen (intention-to-treat analysis). A *P*-value under 0.05 was considered statistically significant.

Results

Thirty-six patients were enrolled in the study. The mean age was 59.6 ± 10.9 y (two females/34 males). There were 18 patients in the group I (supplemented diet) and 18 patients in the control diet group. Characteristics of the patients on enrollment were similar for the two groups, reflecting the homogeneity of the patients. There were no significant differences with regard to gender, mean age, body weight, location and stage of tumour (Table 2). Six patients underwent resection of a tumour located in the oral cavity with unilateral or bilateral neck disection; 30 patients underwent laryngectomy (total or partial) or pharyngo-laryngectomy, with the same distributions of surgery in group I and II. Duration of enteral nutrition in both groups was similar with an average duration of 9.6 ± 9 days.

As shown in Table 3, no significant intergroup differences in the trend of the three plasma proteins and weight were detected.

CRP decreased in both groups (group I: 152.9 ± 76.9 *vs* 68.9 ± 82.5 mg/dl; $P<0.05$; and group II: 105.9 ± 92 *vs* 43.6 ± 59.1 mg/dl; $P<0.05$). IL-6 did not change (group I: 16.3 ± 12.3 *vs* 35.6 ± 83.4 pg/ml; NS; and group II: 22.8 ± 40 *vs* 9.9 ± 17.7 pg/ml; NS). TNFα did not show any changes (group I: 4.6 ± 1.6 *vs* 5.1 ± 1.5 pg/ml; NS; and group II: 8.8 ± 6.1 *vs* 5.8 ± 1.7 pg/ml; NS). Lymphocytes increased in both groups (group I: 1405.6 ± 517 *vs* $1634\pm529\times10^6$/ml; $P<0.05$; and group II: 1355 ± 696 *vs* $1561\pm541\times10^6$/ml; $P<0.05$).

Table 2 Patient characteristics

	Group I	*Group II*
Age (y)	63.1 ± 12.7	59.3 ± 10.5
Men/women	1/17	1/27
Body mass index	26.2 ± 4.7	24.1 ± 4.2
Disease stage		
I	0	0
II	3	4
III	4	5
IV	9	9
Diagnosis of disease		
Oral cavity	3	3
Larynx	15	15

No statistical differences.

Table 3 Visceral serum protein and anthropometric parameters

Parameters	*Basal*	*Day 7*
Albumin (g/dl)		
Group I	2.44 ± 0.5	3.1 ± 0.68*
Group II	2.66 ± 0.5	3.1 ± 0.53*
Prealbumin (mg/dl)		
Group I	14.3 ± 6.4	20.4 ± 7.2*
Group II	12.9 ± 5.4	20.1 ± 6.2*
Transferrin (mg/dl)		
Group I	148.9 ± 42.4	189.1 ± 39.9*
Group II	148.1 ± 53.6	200.1 ± 59.3*
Weight (kg)		
Group I	69.1 ± 13.7	68.8 ± 14.6
Group II	69.2 ± 12.9	68.5 ± 9.4

*$P<0.05$ with basal values.

Discussion

Malnutrition and immunosuppression are two characteristics of head and neck cancer patients (Riboli *et al*, 1996; Bassett & Dobie, 1983). Malnutrition is due to reduced dietary intake secondary to dysphagia, and interleukins secreted by tumour with catabolic action play a dominant role (Todorov *et al*, 1996). Immunosuppression is related to surgery and immunosuppressive capacity of the tumour (Katz, 1983).

There is evidence suggesting that enteral nutrition, supplemented with immunomodulatory agents including arginine, improve immune function and reduce postoperative complications, in different groups of patients such as pancreatic surgery (Di Carlo *et al*, 1999), surgery of stomach and colo-rectum cancer (Gianoti *et al*, 1999; Wu *et al*, 2001), critically ill patients (Caparros *et al*, 2001), and head and neck cancer patients (Riso *et al*, 2000; Van Bokhorst-de van der Schueren *et al*, 2001).

Certain nutrients such as the semi-essential amino acid arginine, RNA and omega 3 fatty acids, may act pharmacologically on the immune system. It has been suggested that these nutrients may improve host immune defences, and specific nutrient substrates seem to act through different mechanisms, although these formulas combine other immunomodulating nutrients and it is difficult to discern what the treatment effect of arginine alone is (Van Bokhorst-de van der Schueren *et al*, 1998; Gianoti *et al*, 1999; Senkal *et al*, 1995).

In our study with an arginine supplemented formula the changes in IL-6 and TNFα were similar in both groups without statistical difference. CRP decreased in both groups and lymphocyte counts increased. Schilling *et al* (1996) did not find significant differences in phagocytic activity of monocytes, activated surface antigen HLA-DR, and CRP levels between a control formula and a formula supplemented with arginine, omega 3 fatty acids and nucleotides; however both formulas were better than a low energy/low fat intravenous solution. However, Taylor *et al* (1999) showed a decrease in CRP levels in head injury patients treated with an enhanced formula without differences in other biochemical parameters. These results were confirmed by Weimann *et al* (1998) in a group of multiple organ failure patients. Perhaps the differences among studies could be due to different treatment of patients and different nutrients in enhanced formulas. It is clear that enteral nutrition is a good option to attenuate the immunosuppression status of these patients (Tagaki *et al*, 2000; Windsor *et al*, 1998; Lin *et al*, 1997), with better results than parenteral nutrition. Arginine stimulates anabolic hormone release, improves survival in gut-derived sepsis by modulating bacterial clearance and accelerates wound healing (Reynolds *et al*, 1988). Fibre-supplemented enteral formulas may have a beneficial effect on components of the gut barrier, as well as the gut mucosa, and on bacterial translocation (Cummings *et al*, 1983). Insoluble fibre, rich in cellulose and lignin, can exert beneficial effects by increasing faecal mass through water absorption, thereby regulating the intestinal transit. Soluble fibres are degraded by anaerobic colonic flora forming short-chain fatty acids, with a trophic effect on the mucosa of the large bowel.

In conclusion, enhanced-formula did not change IL-6 and TNFα levels. Further studies are needed to determine whether route of nutrition or type of formula is the key in why the enhanced-formula failed to show a beneficial effect on IL-6 and TNFα.

References

Barbul A (1990): Arginine and immune function. *Nutrition* **6**, 53–58.
Barbul A, Sisto DA, Wasserkrug HL & Efron G (1990): Arginine enhances wound healing and lymphocyte immune responses in humans. *Surgery* **108**, 331–337.
Bassett MR & Dobie RA (1983): Patterns of nutritional deficiency in head and neck cancer. *Otol. Head Neck Surg.* **91**, 119–125.
Caparros T, Lopez J & Grau T (2001): Early enteral nutrition in critically ill patients with a high protein diet enriched with arginine, fiber and antioxidants compared with a standard high protein diet. The effect on nosocmial infections and outcome. *J. Parent. Enteral Nutr.* **25**, 299–309.
Coghlin-Dickson TM, Wong RM, Offrin RS, Shizuru JA, Johnston LJ, Hu WW & Blume KG (2000): Effect of oral glutamine suplementation during bone marrow transplantation. *J. Parent. Enteral Nutr.* **24**, 61–66.
Cummings JH (1983): Fermentation in the human large intestine: evidence and implications for health. *Lancet* **106**, 1206–1209
Daly JM, Reynolds J & Thom A (1988): Immune and metabolic effects of arginine in the surgical patient. *Ann. Surg.* **208**, 521–523.
Di Carlo V, Gianotti L, Balzano G, Zerbi A & Braga M (1999): Complications of pancreatic surgery and the role of perioperative nutrition. *Dig. Surg.* **16**, 320–326.
Gianoti L, Braga M, Fortis C, Soldini L, Vignali A, Clombo S, Radaelli G & 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. Parent. Enteral Nutr.* **23**, 314–320.
Jones CD, Palmer TE & Griffiths RD (1999): Randomized clinical outcome study of criticaly ill patients given glutamine-supplemented enteral nutrition. *Nutrition* **15**, 108–115.
Katz AE (1983): Immunologic stating of patients with carcinoma of the head and neck. *Laryngoscope* **93**, 445–463.
Kulkarni AD, Fanslow WC, Rudolph FB & Van Buren CT (1987): Modulation of delayed hypersensitivity in mice by dietary nucleotide restriction. *Transplantation* **44**, 847–849.
Lin MT, Saito H, Fukushima R, Inaba T, Fukatsu K, Inoue T, Furukawa S, Han I, Matsuda T & Muto T (1997): Preoperative total parenteral nutrition influences postoperative systemic cytokine responses after colorectal surgery. *Nutrition* **13**, 8–12.
Reynolds JV, Daly JM & Zhang S (1998): Immunomodulatory mechanisms of arginine. *Surgery* **104**, 141–151.
Riboli E, Kaaks R & Esteve J (1996): Nutrition and laryngeal cancer. *Cancer Causes Control* **7**, 147–156.
Riso S, Aluffi P, Brugnani M, Farinetti F, Pia F & Dandrea F (2000): Postoperative enteral immunonutrition in head and neck cancer patients. *Clin. Nutr.* **19**, 407–412.
Schilling J, Vranjes N, Fierz W, Joller H, Gyurech D, Ludwig E, Marathias K & Geroulanos S (1996): Clinical outcome and immunology of postoperative arginine, omega 3 fatty acid, and nucleotide enriched enteral feeding: a randomized prospective comparison with standard enteral and low calorie/low fat i.v. solutions. *Nutrition* **12**, 423–429.
Senkal M, Kemen M, Homann HH, Eickhoff U, Beier J & Zumtobel V (1995): Modulation of postoperative immune response by enteral nutrition with a diet enriched with arginine, RNA, and omega3 fatty acids in patients with upper gastrointestinal cancer. *Eur. J. Surg.* **161**, 115–122.
Takagi K, Yammamori H, Toyoda Y, Nakajima N & Tashiro T (2000): Modulating effects of the feeding route on stress response and endotoxin translocation in severely stressed patients receiving thoracic esophagectomy. *Nutrition* **16**, 355–360.
Taylor SJ, Fettes SB, Jewkes C & Nelson RJ (1999): Prospective, randomized, controlled trial to determine the effect of early enhanced enteral nutrition on clinical outcome in mechanically ventilated patients suffering head injury. *Crit. Care* **27**, 2594–2595.
Todorov P, Cariuk P, McDevitt T, Coles B, Feaon K & Fisdale M (1996): Charaterization of cancer cachectic factor. *Nature* **379**, 739–742.
Van Bokhorst-de van der Schueren MAE, von Blomberg-van-der Flier BME & Riezebos RK (1998): Differences in immune status between well-nourished and malnourished head and neck cancer patients. *Clin. Nutr.* **17**, 107–111.
Van Bokhorst-de van der Schueren MA, Quak JJ, Von Blomberg-van der Flier ME, Kuik DJ, Langendoen SI, Snow GB, Green CJ & van Leeuwen PAM (2001): Effect of perioperative nutrition, with and without arginine supplementation, on nutritional status, immune function, postoperative morbidity, and survival in severely malnourished head and neck cancer patients. *Am. J. Clin. Nutr.* **73**, 323–332.
Weimann A, Bastian L, Bischoff WE, Grotz M, Hansel M, Lotz J, Trautwein C, Tusch G, Schlitt HJ & Regel G (1998): Influence of arginine, omega3 fatty acid and nucleotide supplemented enteral support on systemic inflammatory response syndrome and multiple organ failure in patients after severe trauma. *Nutrition* **14**, 165–172.
Windsor AC, Kanwar S, Li AG, Barnes E, Guthrie JA, Spark JI, Wesh F, Guillou PJ & Reynolds JV (1998): Compared with parenteral nutrition enteral feeding attenauates the acute phase response and improves disease severity in acute pancreatitis. *Gut* **42**, 315–316.
Wu GH, Zhang YW & Wu ZH (2001): Modulation of postoperative immune and inflammatory response by immune enhancing enteral diet in gastrointestinal cancer patients. *World J. Gastroenterol.* **7**, 357–362.