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Anti-oxidant enriched enteral nutrition and immuno-inflammatory response after major gastrointestinal tract surgery

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ABSTRACT

Major surgery induces an immuno-inflammatory response accompanied by oxidative stress that may impair cellular function and delay recovery. The objective of the study was to investigate the effect of an enteral supplement (Module AOX), containing glutamine and anti-oxidants, on circulating levels of immuno-inflammatory markers after major gastrointestinal tract surgery. Patients (n = 21) undergoing major gastrointestinal tract surgery were randomized in a single centre, open label study. The effects on circulating levels of immun0-inflammatory markers were determined on the day before surgery and on days 1, 3, 5 and 7 after surgery. Major gastrointestinal surgery increased IL-6, TNF receptor 55/60 (TNF R55) and C-reactive protein (CRP). Surgery reduced human leukocyte antigen-DR (HLA DR) expression on monocytes. C-reactive protein decrease was more pronounced in the first seven days in the treatment group compared with the control group. In the treatment group, from the moment Module AOX was administered on day one after surgery, TNF receptor 75/80 (TNF R75) level decreased until the third postoperative day and then stabilised, whereas in the control group the TNF receptor 75/80 level continued to increase. The results of the present pilot study suggest that enteral nutrition enriched with glutamine and anti-oxidants possibly moderates the immuno-inflammatory response (CRP, TNF R75) after surgery.

INTRODUCTION

Major surgery induces the production of reactive oxygen species as part of an immuno-inflammatory response that may cause cellular injury by damaging lipids, proteins and DNA. These events may contribute to post-operative complications and organ failure [1-9]. It is known that after major surgery, levels of anti-oxidants such as glutathione, vitamin C, vitamin E, β-carotene, Zn and Se are reduced [7,10-12]. In addition, the lack of anti-oxidant intake in surgical patients with upper gastrointestinal cancer increases susceptibility for oxidative stress-related complications [13-15]. Supplementation of anti-oxidants may support recovery in these patients.

In the present study we report on effects of anti-oxidant enriched enteral nutrition on several circulating and cellular markers of the immuno-inflammatory response in patients after upper gastrointestinal surgery.

PATIENTS AND METHODS

Patients

From February 2002 until May 2003, twenty-one patients who were scheduled for cancer surgery of the oesophagus, stomach, or pancreas, in the VU University Medical Centre (Amsterdam, The Netherlands), were included in the study. Inclusion criteria were: eligibility for jejunostomy feeding, aged 18-75 years, BMI < 35 kg/m2, written informed consent, and a surgical procedure of at least 3 h. Exclusion criteria were: history of cardiovascular or kidney disease, weight loss > 10% in the previous 6 months, steroids or investigational drugs used in the previous 6 weeks and HIV infection. The study was approved by the Medical Ethics Committee of the VU University Medical Centre Amsterdam and conducted according to the Declaration of Helsinki of 1975 (as revised in 1983).

Methods

The study was a prospective, open-label, randomised clinical trial of two balanced groups in parallel design in one medical centre. On the first post-operative day patients were randomized to receive either a standard enteral feeding (Sondalis ISO®; Nestlé, Switzerland), or the same enteral formula in combination with Module AOX (Nestlé, Switzerland). Module AOX provides enrichments in glutamine, cysteine, vitamins C and E, β-carotene, Zn and Se (for precise contents, see Table 1 of van Stijn et al. [16]). The dosage of each component of Module AOX was established with regards to safety

aspects, relying on outcomes of studies on the separate components. Daily intake was calculated by weighing the feeding bags before and after administration.

A jejunostomy feeding tube was placed preoperatively and continuous feeding was started on the first post-operative day. Patients received two Modules AOX per day when feeding could be increased beyond 500 mL/day. Module AOX was administered for a minimum of 5 days and a maximum of 7 days. The feeding schedule was adjusted according to the energy requirements with the goal of reaching 1500-2000 mL from the third postoperative day of the patient. Patients were not allowed to receive additional vitamins, amino acids or lipid solutions during the study period. Daily fluid requirements were given intravenously, starting directly after surgery, until oral intake was sufficient. This fluid also contained glucose.

We measured C-reactive protein (CRP), peripheral leucocyte count, human leucocyte antigen-DR (HLA-DR) expression on the monocytes, soluble IL-1 receptor II, IL-6, IL-8, elastase, bactericidal/permeability-increasing protein (BPI), soluble TNF receptor 55/60 (TNF-R55) and soluble TNF receptor 75/80 (TNF-R75). Blood samples were taken on the day before surgery (day -1) and on days 1, 3, 5 and 7 after surgery. The samples on day 1 were taken before the start of the enteral nutrition. All samples were taken between 08.00 and 10.00 hours a.m..

Preparation, storage and analysis of blood samples

After sampling, several sensitive samples were immediately put on melting ice, such as for IL-1 receptor II, IL-6, IL-8, BPI, TNF-R55 and TNF-R75, to avoid degradation. Plasma was prepared by a two-step centrifugation procedure: 1400 g, 4°C, for 10 min, followed by 2700 g, 4°C, for 10 min, and stored at -80°C.

IL-1 receptor II. The human soluble IL-1 receptor II ELISA kit (Hycult Biotechnology B.V., Uden, The Netherlands) was used to determine the concentration of IL-1 receptor II, representing IL-1 in plasma.

IL-6. IL-6 was measured with a commercially available automated solid-phase, two-site two-step chemiluminescent immunometric assay (Immulite®; DPC, Los Angeles, CA, USA).

IL-8. IL-8 was determined using specific sandwich ELISA as described previously [17].

Bactericidal/permeability-increasing protein. Plasma was spun twice before storage to prevent leucocyte contamination of plasma. Microtitre plates were coated with human BPI-specific monoclonal antibody 4E3. Washing and dilution buffers for BPI determination contained 80mM-$MgCl_2$. Mg^{2+} ions were added to prevent any influence of endotoxin on the BPI measurement. Human recombinant BPI (provided by M. Marra, InCyte, Palo Alto, CA, USA) was used for the standard curve. Diluted samples (1:2 for BPI) were assayed. Biotinylated polyclonal rabbit anti-human BPI IgG was used as the

secondary antibody, followed by visualisation using peroxidase-conjugated streptavidin (Dakopatts, Glostrup, Denmark). The level of detection was 200 pg/mL [18].

TNF receptor 55/60 and TNF receptor 75/80. Monoclonal antibodies specifically directed against TNF-R55 and TNF-R75 were obtained as described elsewhere [19]. Polyclonal rabbit antisera, anti-TNF-R55 and anti-TNF-R75 were obtained by immunising rabbits with TNF-R55 and TNF-R75, respectively. We used the monoclonal antibodies that were kindly provided by Dr R. Devos (Hoffmann La-Roche, Welwyn Garden City, Herts, UK).

C-reactive protein. Plasma levels of CRP were measured by an immunoturbidimetric method, using a Hitachi 747 analyser (Roche Diagnostics, Mannheim, Germany).

Leucocytes. Leucocytes were measured using a Sysmex SE9000 analyser (Sysmex Corporation, Kobe, Japan).

Human leucocyte antigen-DR expression. HLA-DR antigen expression was measured in fresh heparinised venous blood after lysis of erythrocytes within 1 hour after blood sampling (Q prep; Coulter Corp., Miami, FL, USA). The absolute numbers of leucocytes and the percentage of monocytes ($CD14^+$) were determined. The expression of the HLA-DR antigen on $CD14^+$ cells was evaluated by FACS analysis (FACStar Plus; Becton Dickinson, San Jose, CA, USA) and was expressed as mean channel fluorescence intensity as described previously [20].

Elastase. Serum levels of elastase were determined by immunoassay (Merck, Darmstadt, Germany) according to the manufacturer's instructions.

Statistical analysis

The interval and ratio variables are expressed as means and standard deviations. The Mann-Whitney U test was performed to analyse patient characteristics, tumour characteristics and the difference between the treatment group and the control group in change over time. Fischer's exact test was performed to analyse the nominal variables of the patient and tumour characteristics. Differences between the control and treatment group in the course of the post-operative immuno-inflammatory response were analysed using general estimating equations (GEE). GEE-analysis is a linear regression technique and is comparable with the mixed model factorial ANOVA. However, none of the disadvantages of the mixed-model factorial ANOVA (for example, no missing data allowed, equal time intervals assumed, no 'real' effect measures) is present in GEE-analysis [21].

The Mann-Whitney U test and Fischer's exact test were performed with SPSS 14.0 for Windows® (SPSS, Inc., Chicago, IL, USA). GEE-analysis was performed with STATA® (version 7.0; StataCorp LP, College Station, TX, USA) [21]. For all analyses, P<0.05 was considered statistically significant.

RESULTS

In total, twenty-seven patients were considered eligible for enrolment in the study, of which eleven patients were included in the control group and ten patients in the treatment group. Six patients did not meet the inclusion criteria and were therefore excluded from the study. In the control group one patient died on the second day after surgery, before receiving any enteral feeding. Another patient in the control group refused further blood sampling. According to the principle of 'intention-to-treat analysis', results of all twenty-one patients were analysed. The control and treatment group were comparable with respect to anthropometrics, surgery and tumour characteristics (see Tables 2 and 3 in van Stijn et al. [16]).

Intake
Patients reached an average 60% of their daily energy expenditure, and there were no differences between the two groups (control group: 1944 (SD 133) kcal (8134 (SD 556) kJ); treatment group: 1750 (SD 113) kcal (7322 (SD 473) kJ)) (see Figure 1 in van Stijn et al. [16]).

Immuno-inflammatory response
Most immuno-inflammatory markers showed no differences between the groups. Therefore only CRP, IL-6, TNF-R75 and HLA-DR are shown in Figure 1. In both groups an expected rise in CRP up to 3 days was followed by a decrease. The course of CRP over time was significantly lower in the treatment group (P=0.04; Figure 1a). The levels of IL-6 (Figure 1b), IL-1, IL-8 and TNF-R55 showed similar courses but no differences were found between the control and treatment groups. A steady increase in TNF-R75 levels was found in the control group during the first 7 d after surgery (P=0.001; Figure 1c). During the first three post-operative days, TNF-R75 levels behaved significantly different in the treatment group in change over time compared with the control group (P=0.04); levels continued to rise in the control group, whereas in the treatment group they decreased until the third post-operative day and then stabilised. HLA-DR expression was reduced in both groups (Figure 1d). No significant differences between the groups were found for leucocyte, BPI and elastase levels.

DISCUSSION

Major surgical procedures are frequently associated with tissue damage and ischaemia–reperfusion injury, resulting in oxidative stress and deterioration of the immune response. After major surgery, anti-oxidant levels are low and antioxidant supplementation may

help to moderate the inflammatory response in an attempt to improve post-operative course [1,2]. In the present prospective study, an enteral anti- oxidant formula (Module AOX) was studied in patients undergoing major upper gastrointestinal surgery.

The surgical trauma was reflected in the rise of the proinflammatory mediators CRP, IL-6, and TNF-R55 in both groups (Figure 1a-c). After surgery, HLA-DR expression was significantly reduced, indicating an injury-related cellular immune deficiency, which is consistent with other reports (Figure 1) [1,22,23].

Figure 1. Development of immuno-inflammatory markers in control (- - -) and treatment (Module AOX; —) groups:
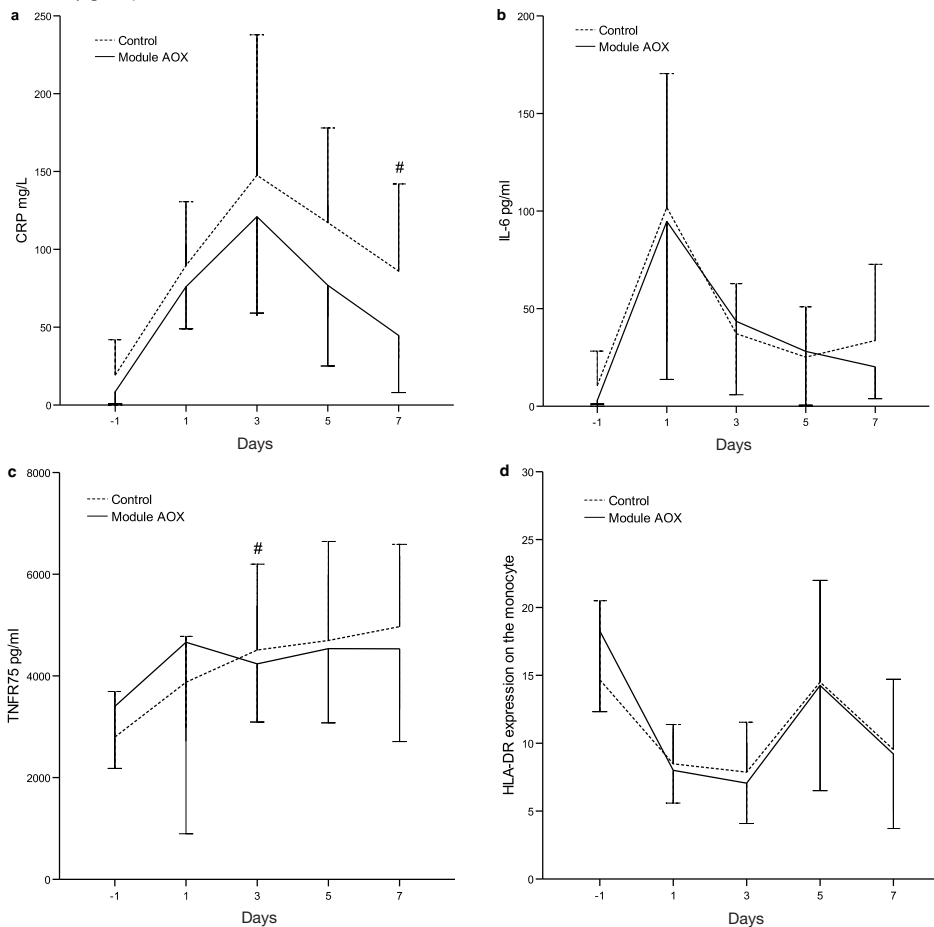
a. C-reactive protein (CRP); b. IL-6; c. TNF receptor 75/80 (TNF-R75); d. human leucocyte antigen-DR (HLA-DR). Values are means, with standard deviations represented by vertical bars. * Statistically significant difference between control group and treatment group in change over time, corrected for baseline (P<0.05).

CRP is produced rapidly and at high levels, by the liver, during acute-phase reactions [24]. CRP recognises pathogens by ligand binding which efficiently activates the classical complement cascade [24,25]. Module AOX had a moderating effect on the course of CRP production after surgery. After the initial rise in CRP level up to day 3 the decrease in CRP levels was more pronounced in the Module AOX group.

Major surgery induces the production of TNF-a, a cytokine that activates the innate immune system, by induction of other cytokine production, activation and expression of adhesion molecules, and growth stimulation. This potent biological activity of TNF can be harmful when not adequately controlled and can lead to systemic inflammatory response syndrome [2,26]. TNF-a is a ligand for the soluble TNF receptors TNF-R55 and TNF-R75 that are markers of the pro-inflammatory response and responsible for inactivation and clearance of TNF [27,28]. In trauma patients who received enteral nutrition enriched with glutamine, reduced TNF-R75 levels were associated with lower infectious morbidity [29].

In the present study, from the moment Module AOX was administered at day 1, TNF-R75 levels decreased until the third post-operative day and then stabilised, whereas in the control group, TNF-R75 levels increased during the entire post-operative period. This seems to suggest that hyperactivity of the immune response might be moderated by administration of Module AOX.

Limitations of the study

The present pilot study was part of a feasibility study evaluating the safety and tolerance of Module AOX, which included only a small group of patients. Therefore, our findings on the inflammatory markers need to be interpreted with caution. The more severely ill and malnourished patients were not included, by using the 10% 6-month weight loss as an exclusion criterion. It is known that better clinical effects in nutritional intervention studies can be expected in patients with a greater severity of illness and malnutrition, using the NRS-2002 (Nutritional Risk Screening) as the nutritional assessment [30]. Although the study was not strongly powered, significance was still reached for several immuno-inflammatory markers, despite the fact that more severely ill and malnourished patients were excluded. A more pronounced result can be expected with this kind of nutrition in severely ill and malnourished patients.

Conclusions

In conclusion, the present study shows that enteral nutrition enriched with glutamine and anti-oxidants is a safe and well-tolerated supplement to standard enteral nutrition. Major surgery induces a systemic immuno-inflammatory response with reduced HLA-DR expression and increased concentrations of CRP, IL-6 and TNF-R75. Enteral nutrition enriched with glutamine and anti-oxidants possibly moderates the immuno-inflammatory

response (CRP, TNF-R75) after surgery. Larger studies are needed to reproduce these results and to investigate the effects of Module AOX on morbidity.

REFERENCES

1.   Park SK, Brody JI, Wallace HA, et al. (1971) Immunosuppressive effect of surgery. Lancet i, 53-55.

2.   Decker D, Tolba R, Springer W, et al. (2005) Abdominal surgical interventions: local and systemic consequences for the immune system - a prospective study on elective gastrointestinal surgery. J Surg Res 126, 12-18.

3.   Delves PJ & Roitt IM (2000) The immune system. First of two parts. N Engl J Med 343, 37-49.

4.   Anup R, Aparna V, Pulimood A, et al. (1999) Surgical stress and the small intestine: role of oxygen free radicals. Surgery 125, 560-569.

5.   Thomas S, Prabhu R & Balasubramanian KA (2005) Surgical manipulation of the intestine and distant organ damage - protection by oral glutamine supplementation. Surgery 137, 48-55.

6.   Bautista AP, Schuler A, Spolarics Z, et al. (1991) Tumor necrosis factor-a stimulates superoxide anion generation by perfused rat liver and Kupffer cells. Am J Physiol 261, G891-G895.

7.   Heyland DK, Dhaliwal R, Suchner U, et al. (2005) Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient. Intensive Care Med 31, 327-337.

8.   Cadenas S & Cadenas AM (2002) Fighting the stranger - antioxidant protection against endotoxin toxicity. Toxicology 180, 45-63.

9.   Nathens AB, Neff MJ, Jurkovich GJ, et al. (2002) Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. Ann Surg 236, 814-822.

10.   Manhart N, Vierlinger K, Spittler A, et al. (2001) Oral feeding with glutamine prevents lymphocyte and glutathione depletion of Peyer's patches in endotoxemic mice. Ann Surg 234, 92-97.

11.   Boelens PG, Nijveldt RJ, Houdijk AP, et al. (2001) Glutamine alimentation in catabolic state. J Nutr 131, Suppl. 9, 2569S-2577S.

12.   Berger MM & Shenkin A (1998) Trace elements in trauma andburns. Curr Opin Clin Nutr Metab Care 1, 513-517.

13.   Gurski RR, Schirmer CC, Rosa AR, et al. (2003) Nutritional assessment in patients with squamous cell carcinoma of the esophagus. Hepatogastroenterology 50, 1943-1947.

14.   Kruizenga HM, Wierdsma NJ, van Bokhorst MA, et al. (2003) Screening of nutritional status in The Netherlands. Clin Nutr 22, 147-152.

15.   Xu J, Zhong Y, Jing D, et al. (2006) Preoperative enteral immunonutrition improves postoperative outcome in patients with gastrointestinal cancer. World J Surg 30, 1284-1289.

16.   van Stijn MF, Ligthart-Melis GC, Boelens PG, et al. (2008) Antioxidant enriched enteral nutrition and oxidative stress after major gastrointestinal tract surgery. World J Gastroenterol 14, 6960-6969.

17.   Dentener MA, Bazil V, Von Asmuth EJ, et al. (1993) Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-a, IL-6 and IL-8 release by human monocytes and alveolar macrophages. J Immunol 150, 2885-2891.

18.   Hiki N, Berger D, Mimura Y, et al. (2000) Release of endotoxin-binding proteins during major elective surgery: role of soluble CD14 in phagocytic activation. World J Surg 24, 499-506.

19. van Dielen FM, van't Veer C, Schols AM, et al. (2001) Increased leptin concentrations correlate with increased concentrations of inflammatory markers in morbidly obese individuals. Int J Obes Relat Metab Disord 25, 1759-1766.

20. Boelens PG, Houdijk AP, Fonk JC, et al. (2002) Glutamine enriched enteral nutrition increases HLA-DR expression on monocytes of trauma patients. J Nutr 132, 2580-2586.

21. Twisk JWR (2003) Applied Longitudinal Data Analysis for Epidemiology. A Practical Guide. Cambridge, UK: Cambridge University Press.

22. Tashiro T, Yamamori H, Takagi K, et al. (1999) Changes in immune function following surgery for esophageal carcinoma. Nutrition 15, 760-766.

23. Spittler A, Razenberger M, Kupper H, et al. (2000) Relationship between interleukin-6 plasma concentration in patients with sepsis, monocyte phenotype, monocyte phagocytic properties, and cytokine production. Clin Infect Dis 31, 1338-1342.

24. Black S, Kushner I & Samols D (2004) C-reactive protein. J Biol Chem 279, 48487-48490.

25. Marnell L, Mold C & Du Clos TW (2005) C-reactive protein: ligands, receptors and role in inflammation. Clin Immunol 117, 104-111.

26. Hehlgans T & Pfeffer K (2005) The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology 115, 1-20.

27. el Barbary M & Khabar KS (2002) Soluble tumor necrosis factor receptor p55 predicts cytokinemia and systemic inflammatory response after cardiopulmonary bypass. Crit Care Med 30, 1712-1716.

28. Bemelmans MH, van Tits LJ & Buurman WA (1996) Tumor necrosis factor: function, release and clearance. Crit Rev Immunol 16, 1-11.

29. Houdijk AP, Rijnsburger ER, Jansen J, et al. (1998) Randomised trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma. Lancet 352, 772-776.

30. Kondrup J, Rasmussen HH, Hamberg O, et al. (2003) Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. Clin Nutr 22, 321-336.