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RAPID COMMUNICATION

Antioxidant enriched enteral nutrition and oxidative stress after major gastrointestinal tract surgery

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Abstract

AIM: To investigate the effects of an enteral supplement containing antioxidants on circulating levels of antioxidants and indicators of oxidative stress after major gastrointestinal surgery.

METHODS: Twenty-one patients undergoing major upper gastrointestinal tract surgery were randomised in a single centre, open label study on the effect of postoperative enteral nutrition supplemented with antioxidants. The effect on circulating levels of antioxidants and indicators of oxidative stress, such as F2-isoprostane, was studied.

RESULTS: The antioxidant enteral supplement showed no adverse effects and was well tolerated. After surgery a decrease in the circulating levels of antioxidant parameters was observed. Only selenium and glutamine levels were restored to pre-operative values one week after surgery. F2-isoprostane increased in the first three postoperative days only in the antioxidant supplemented group. Lipopolysaccharide binding protein (LBP) levels decreased faster in the antioxidant group after surgery.

CONCLUSION: Despite lower antioxidant levels there was no increase in the circulating markers of oxidative stress on the first day after major abdominal surgery. The rise in F2-isoprostane in patients receiving the antioxidant supplement may be related to the conversion of antioxidants to oxidants which raises questions on antioxidant supplementation. Module AOX restored the postoperative decrease in selenium levels. The rapid decrease in LBP levels in the antioxidant group suggests a possible protective effect on gut wall integrity. Further studies are needed on the role of oxidative stress on outcome and the use of antioxidants in patients undergoing major abdominal surgery.

Key words: Antioxidants; Critical illness; Enteral nutrition; Oxidative stress; Surgery; Upper gastrointestinal tract

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INTRODUCTION

Major surgery and critical illness induce an immuno-

inflammatory response, which is accompanied by the production of reactive oxygen species (ROS) at the site of injury[1-5]. Oxidative stress is defined as a state in which the level of ROS exceeds the endogenous antioxidant defences of the host. Reactive oxygen species can cause direct cellular injury by damaging lipids, proteins and DNA. This might result in tissue injury and organ dysfunction. Therefore, oxidative stress probably plays a key role in the development of organ failure[6-12]. In situations of major surgery and critical illness, a redistribution of antioxidants occurs to tissues or organs in need. This results in a depletion of antioxidant stores that may be deleterious when oxidative stress is prolonged[13]. In these situations the supplementation of certain antioxidant amino acids (glutamine, cysteine) and antioxidant micronutrients (zinc, Vitamin C, Vitamin E, β-carotene, selenium) may improve outcome. There is little information on the effect of major abdominal surgery and antioxidant supplementation on the blood levels of parameters of antioxidant capacity and oxidative stress.

To protect the host from oxidative stress, humans have an extensive antioxidant defence system consisting of enzymatic and non-enzymatic factors. Enzymes which are involved in antioxidant function are superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Superoxide dismutase catalyzes dismutation of the superoxide anion into hydrogen peroxide[14]. Glutathione peroxidase reduces hydrogen peroxide and organic hydroperoxides into water or alcohol, and reverts two glutathione (GSH) molecules to glutathione disulfide (GSSG)[14,15]. For GSH-Px and SOD function, the trace elements selenium and zinc are important, respectively[16].

Among the non-enzymatic factors, alpha tocopherol (Vitamin E), Vitamin C, β-carotene, and GSH function as antioxidants. Vitamin E is the main fat-soluble antioxidant in humans. Vitamin E scavenges peroxyl radicals, produced during lipid peroxidation, which leads to a tocopherol radical[17,18]. Vitamin C, a potent intra- and extracellular antioxidant[19], scavenges superoxide, hydroxyl and peroxyl radicals, and reacts with hypochlorite and singlet oxygen[15]. β-carotene is a precursor of Vitamin A. The conjugated double-bonds of β-carotene are able to open and scavenge singlet molecular oxygen and peroxyl radicals[20]. Glutathione is an intracellular antioxidant and a co-enzyme of GSH-Px. Glutamine and cysteine are precursors of the antioxidant GSH[15]. Furthermore, selenium, GSH, Vitamin E and Vitamin C function synergistically to regenerate both water and fat-soluble antioxidants[10]. The extent of oxidative stress can be measured by determining the end-products of lipid peroxidation; malondialdehyde (MDA) and F_2-isoprostane[15,21,22].

The present study investigates the effect of antioxidant-supplemented enteral feeding on circulating factors of the antioxidant defence system and markers of oxidative stress in patients after major upper gastrointestinal tract surgery.

MATERIALS AND METHODS

Patients

From February 2002 until May 2003, twenty-one patients undergoing elective surgery of the oesophagus, stomach or pancreas, in the VU University Medical Center (Amsterdam, The Netherlands), were included in the study. Inclusion criteria were: eligible for jejunostomy feeding, between 18 and 75 years old, body mass index (BMI) below 35, written informed consent, and a surgical procedure of at least three hours. Exclusion criteria were: history of cardiovascular or kidney disease, weight loss of > 10% in last six months, steroids or investigational drug used in the last six weeks and human immunodeficiency virus (HIV) infection. The study was approved by the Ethics Committee of the VU University Medical Centre and conducted according to the Declaration of Helsinki.

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 day after surgery, patients were randomly assigned to the control group and were started on standard tube feeding (Sondalis ISO®, Nestlé, Switzerland), or the treatment group, and were started on the same tube feeding in combination with Module AOX (Nestlé, Switzerland). Sondalis ISO® is a nutritionally balanced, complete liquid diet. Module AOX is a plastic unit containing powder which contains per unit 37 kcal, 7.4 g protein, 0.04 g lipid and 1.9 g carbohydrate. The contents of Sondalis ISO® and Module AOX are listed in Table 1. The dosages of the compounds in Module AOX were established with respect to safety aspects. One Module AOX unit was added to one pouch of Sondalis ISO® (500 mL), with a maximum intake of two devices of Module AOX per day. Module AOX was upstream connected to the enteral feeding pouch. After mixing with enteral feeding, the module was connected to the administration set and was ready for immediate consumption by the patient. Feeding pouches were weighed before the start of feeding and after being discarded. This made it possible to calculate exactly the daily intake of kcal and nutritional compounds.

Feeding was administered by a jejunostomy feeding tube and started on the first day after surgery. In the treatment group, Module AOX was added to the enteral feeding from the first day after surgery. Patients received two Modules AOX per day when feeding could be increased beyond 500 mL per day. Module AOX was administered for a minimum of five and a maximum of seven days. Patients were fed continuously. The intention was to give the patient 500 mL of feeding on the first day after surgery, 1000-1500 mL on the second day and 1500-2000 mL from the third day. The feeding schedule was adjusted according to the energy requirements of the patient, which was established using indirect calorimetry. Oral food intake was allowed from day five after the

Table 1 Contents of Sondalis ISO® and Module AOX

	Sondalis ISO® per 100 mL	Module AOX per unit
kcal	100	37
Protein (g)	3.8	7.4
Glutamine (g)	0.34	6
Cysteine (g)	0.03	2.5
Vitamin C (mg)	5.4	140
Vitamin E (mg)	1	30
B-carotene (mg)	0	6
Zinc (mg)	1	6
Selenium (μg)	4.4	50

start of tube feeding. Patients were not allowed to receive additional Vitamins, amino acids or lipid solutions during the study period.

Endpoints of the study

The present study was part of a larger study testing the safety and tolerance of the supplemented ingredients. The effect of the intake of antioxidants (Module AOX, Nestlé, Switzerland) on indicators of oxidative stress was studied in a group of patients undergoing major elective surgery.

Levels of the administered nutritional compounds, indicators of oxidative stress after surgery and indicators of the inflammatory response after surgery were measured in plasma, serum and urine. F2-isoprostane (in urine) and malondialdehyde (MDA, in serum) were measured as parameters of oxidative stress. Total cysteine, Vitamin C, Vitamin E, β-carotene, zinc, selenium and GSH-Px were measured as parameters of antioxidant/oxidant status. Peripheral white blood cell count (WBC), Interleukin 6 (IL-6) and lipopolysaccharide-binding protein (LBP) were measured as parameters of surgery-induced inflammatory response.

Blood and urine samples were taken on the day before surgery (-1) and on day one (1), three (3), five (5) and seven (7) after surgery. The samples on day one (1) were taken before the start of enteral nutrition. All samples were taken between eight and ten a.m.

Preparation, storage and analysis of samples

Amino acids: Blood was collected on heparin. Plasma was separated immediately from the blood by centrifugation (2000 g) at 4℃ for ten minutes. Five hundreds μL of plasma was added to tubes containing 20 mg of solid sulfosalicyclic acid for deproteinizing, vortex mixed and subsequently stored at -80℃ until analysis. The concentration of glutamine was determined by reversed-phase high-performance liquid chromatography as previously described[23].

Cysteine: Blood was collected on heparin. Plasma was separated immediately from the blood by centrifugation (2000 g) at 4℃ for four minutes. Aliquots containing 500 μL of plasma were stored at -80℃ until analysis. Total cysteine concentrations were measured according to Malloy *et al*[24].

Vitamin E and β-carotene: Blood was collected in a serum separation tube. Blood was centrifuged (2000 g) at 20℃ for ten minutes and serum was stored at -80℃ until analysis. Serum vitamin E and β-carotene were determined as essentially described by Miller and Yang[25].

Vitamin C: Blood was collected in tubes containing EDTA (ethylenediaminetetraacetic acid). Plasma was separated immediately from the blood by centrifugation (1400 g) at 4℃ for ten minutes. Plasma was further purified by centrifugation (2700 g) at 4℃ for ten minutes. For the determination of total vitamin C (the sum of ascorbic acid and dehydroascorbic acid) plasma was stabilized by the addition of metaphosphoric acid[26] and stored at -80℃ until analysis. After deproteinization and enzymatic oxidation of ascorbic acid to dehydroascorbic acid, the latter was condensed with ortho-phenylenediamine to its derivative. This derivative was separated by reversed-phase high-performance liquid chromatography with fluorescence detection[27]. The between-assay coefficient of variation was < 4%.

Zinc and selenium: Serum was separated from the blood by centrifugation (2000 g) at 20℃ for ten minutes. Tubes cleaned with mineral-free water, were used to store serum at -80℃ until analysis. Zinc and selenium were determined by Zeeman corrected atomic absorption spectrometry. The flame was used for zinc determination. A graphite furnace and Palladium-modifier were used for the determination of selenium.

F2-Isoprostane [8-iso-Prostaglandin (PG) $F_{2\alpha}$]: Urine was collected in plastic tubes and stored at -80℃ until analysis. Urine 8-iso PGF 2α concentrations were determined using LC-MS/MS. Prior to analysis, the urine samples were thawed, mixed and centrifuged. Subsequently 0.1 mL of the labelled internal standard (10 ng/mL; 8-iso PGF 2α-d4, Cayman cat. 316350) was added to 1 mL of urine. The 'sample clean up procedure' was performed according to the method described by Bohnstedt *et al*[28]. Thereafter the samples were redissolved in 0.1 mL of 10% acetonitrile and 40 μL was injected into a Waters X-terra MS C18 column (3.5 μm, 2.1 mm × 100 mm; cat. 186000404) connected to a Quattro Micro (Waters, Milford, MA, USA) triple quadruple mass spectrometer running in negative electrospray ionization mode. Separation took place using a gradient from 7% to 33% acetonitrile, containing 0.3% ammonia. With this gradient the internal standard (357.2 > 197.3 amu) and 8-iso PGF 2α (353.2 > 193.3 amu) eluted at approximately nine minutes. The peak areas were then integrated and the ratios were calculated. The unknown samples were compared with a standard/internal standard calibrator (50 and 10 ng/mL, respectively) in order to calculate the 8-iso PGF 2α concentration in the urine samples. The within and between assay coefficients of variation were < 8% and < 9%, respectively. Finally, 8-iso PGF 2α was calculated in ng per mg creatinine in urine.

MDA: Serum was separated from the blood by centrifugation (2000 g) at 20℃ for ten minutes and stored at

-80℃ until analysis. Serum MDA concentrations were determined by high-performance liquid chromatography with fluorescence detection as described by van de Kerkhof *et al*[29].

GSH-Px: GSH-Px activity was determined in red cell hemolysate, left behind after separation of EDTA plasma and stored at -80℃. GSH-Px was measured as described by Karsdorp *et al*[30], using an Elan analyser (Merck, Germany).

WBC: WBC was measured using a Sysmex SE9000 analyzer (Sysmex Corporation, Kobe, Japan).

IL-6: IL-6 was measured with a commercially available automated solid-phase, two-site, two-step chemiluminescent immunometric assay according to the specifications of the manufacturer (Immulite®; DPC, Los Angeles, CA, USA). This assay employs a murine mAb against the IL-6 (capture antibody) and a polyclonal anti-IL-6-detecting antibody. The values were expressed in pg/mL based on the reference standard supplied by the manufacturer, with limits of detection between 2 and 1000 pg/mL.

LBP: LBP was measured in EDTA plasma with a commercially available automated solid-phase, two-site, two-step chemiluminescent immunometric assay according to the specifications of the manufacturer (Immulite®; DPC, Los Angeles, CA, USA). This assay employs a murine mAb against the LBP (capture antibody) and a polyclonal anti-LBP-detecting antibody. The values were expressed in μg/mL based on the reference standard supplied by the manufacturer, with the limits of detection between 0.2 and 200 μg/mL.

Statistical analysis

The interval/ratio variables were expressed as mean and standard deviation. The Mann-Whitney *U* test was performed to analyse patient characteristics, the tumour characteristics and the difference between the control and treatment group over time. Fischer's Exact test was performed to analyse the nominal variables of the patient and tumour characteristics. The Wilcoxon Signed Ranks Test was performed per group of patients to obtain the effect of the surgical intervention between the day before surgery (-1) and the first postoperative day (1). Differences between the control and the treatment group in the development of postoperative antioxidant and oxidant parameters were analysed using the general estimating equations (GEE) population averaged model. GEE is a linear regression technique, which is suitable for analysing results from a longitudinal study in which outcome variables are repeatedly measured in each individual[31]. Time is treated as a categorical variable, represented by dummies. In a single analysis the differences between the treatment and control group over time were analysed, corrected for baseline. The GEE analysis was performed following corrections for gender, age, smoking, chemotherapy before surgery, surgery (open or laparoscopic), duration of surgery, blood loss, tumour size or intake of calories. The Wilcoxon Signed Ranks test, the Mann-Whitney *U* test and the 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)[31]. For all analyses a *P*-value < 0.05 was considered significant.

RESULTS

No side-effects were observed following the administration of Module AOX. With regard to practicalities, the set-up of Module AOX was not found to be difficult by any of the nurses involved in the study. No serious events, such as occlusion of the tube were observed. As for tolerance, the daily weight of stools was not different between the control and the treatment group, the consistency of stools (liquid or soft, formed or hard) did not differ between the groups, nor did abdominal pain, but flatulence was less intense in the treatment group (data not shown).

Patient characteristics and follow-up

In total 27 patients were considered eligible for enrolment in the study, of which eleven were included in the control group and ten in the treatment group. Six patients were excluded from the study before randomization because no jejunostomy was available, which was necessary for the feeding route. In the control group, one patient died on the second day after surgery, before receiving enteral feeding. Another patient in the control group refused further blood sampling. According to the principle of 'intention to treat analysis', the results of all 21 patients were analyzed.

Patients received upper gastrointestinal tract surgery. No differences in the occurrence of postoperative complications, infectious and non-infectious, were found between the control and treatment group in the first week after surgery. Patients in the control group stayed an average of 2.5 ± 0.7 d and patients in the treatment group stayed an average of 3.7 ± 0.8 d on an intensive or medium care unit ($P = 0.236$). Baseline patient characteristics and tumour characteristics are given in Tables 2 and 3, respectively. The control and treatment groups were comparable with respect to anthropometrics, surgery and tumour characteristics.

Intake

Intake of Sondalis ISO® with or without Module AOX was started as soon as possible after surgery. Caloric intake was based on caloric requirements. However, the patients reached on average 60% of their daily caloric requirements, as measured on the day before surgery. In both groups, patients received a similar amount of calories during the treatment period (Figure 1). A plateau in intake was reached in both groups between three and five days after surgery. Table 4 shows the absolute intake of nutrients in the control and the treatment group. The

Table 2 Patient characteristics

	Control group $n = 11$	Treatment group $n = 10$	*P*-value
Male/Female	9/2	6/4	0.361[1]
Age (yr)	62 (8)	57 (10)	0.230[2]
Smoking/not smoking	3/8	6/4	0.198[1]
Bodyweight (kg)	74 (19)	67 (12)	0.245[2]
Height (cm)	174 (5)	174 (12)	0.697[2]
Body Mass Index (kg/m^2)	24 (5)	22 (3)	0.181[2]
Albumin (g/L)	38 (5)	39 (5)	0.426[2]
Chemotherapy prior to surgery (Yes/No)	4/7	5/5	0.670[1]
Surgery type (laparoscopic/open)	4/7	2/8	0.635[1]
Duration of surgery (min)	337 (100)	353 (155)	0.888[2]
Blood loss during surgery (mL)	2168 (1726)	1720 (1232)	0.647[2]
Admission ICU/MCU (d)	2.5 (0.7)	3.7 (0.8)	0.236[2]

[1]Fischer's Exact Test; [2]Mann-Whitney *U* Test. Data expressed as mean (standard deviation).

Table 3 Tumour characteristics (mean)

	Control group	Treatment group	*P*-value
Tumour size (cm)	5.93 ($n = 11$)	5.11 $n = 9$[2]	0.970[4]
Positive lymph nodes (number if present)	1.36 ($n = 7$)	3.00 $n = 5$[1]	0.412[4]
Metastasis (observed during surgery/post-surgery histology)	27.3% ($n = 11$)	30% ($n = 10$)	1.000[3]

[1]One patient had 10 positive lymph nodes; [2]No information on one patient; [3]Fischer's Exact Test; [4]Mann-Whitney *U* Test.

treatment group consumed significantly more glutamine, cysteine, zinc, selenium, Vitamin C, Vitamin E and β-carotene than the control group ($P < 0.001$).

First postoperative day

Significant decreases in the levels of antioxidants were noticed in both groups on the first day after surgery (Table 5). Total cysteine was also reduced significantly by surgery in both groups on the first day after surgery (Table 5). GSH-Px, F2-isoprostane (Figure 2) and MDA were not affected on the first day after surgery in both groups. As for the inflammatory markers, IL-6 and LBP increased in both the control and treatment group. WBC count increased in the treatment group only (Table 5).

Levels of oxidative stress parameters when starting with Module AOX or standard nutrition

Changes in plasma or serum levels of the oxidative stress parameters and the antioxidant/oxidant parameters are shown in Table 6. Glutamine increased significantly in both groups ($P = 0.001$). Total cysteine increased after surgery in both groups ($P < 0.001$). Vitamin E increased in both groups ($P < 0.001$) and Vitamin C increased in the treatment group only ($P = 0.014$). Zinc increased significantly in both groups ($P < 0.001$), as well as selenium ($P = 0.003$), with a greater rise in the treatment group ($P = 0.006$). Only selenium and glutamine preoperative levels were attained one week after surgery. For all other parameters, the levels were below preoperative values after one week. During the first three days after surgery F2-isoprostane significantly increased in the treatment group compared with the control group ($P = 0.007$) (Figure 2). MDA did not change after surgery in both groups. GSH-Px eventually decreased in the control group ($P = 0.013$), as well as in the treatment group. IL-6 tended to decrease after surgery in both groups ($P = 0.062$). No change was observed in WBC after surgery, in either group. Lipopolysaccharide-binding protein showed a peak value at day three in the control group that was significantly higher than that in the treatment group ($P = 0.018$). In the treatment group LBP levels remained at the level of the first postoperative day (Figure 3).

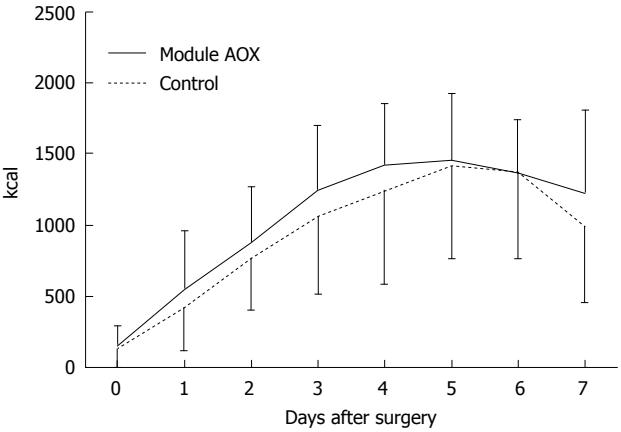

Figure 1 Intake of Kcal after surgery (mean ± SD). Caloric intake on the day before and on 7 d after surgery in the control and treatment groups. No significant difference in intake was observed between the groups.

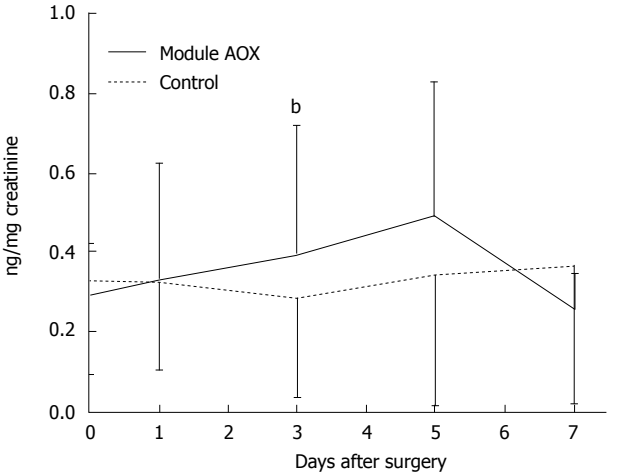

Figure 2 F2-Isoprostane after surgery (mean ± SD). Development of F2-isoprostane in urine (ng/mg creatinine) after surgery in the control and treatment groups. The difference between the two groups over time was observed to be significant between the first and the third day after surgery, $^bP < 0.01$.

DISCUSSION

The present study reports on the effects of an antioxidant

Table 4 Intake of anti-oxidant nutrients per day (mean)

		Days after surgery						
		1	2	3	4	5	6	7
Glutamine (g)	Control	1.2	2.5	3.4	3.9	4.3	4.1	3.2
	Treatment	4.5	7.9	11	11	12	11	7.4
Cysteine (g)	Control	0.1	0.2	0.3	0.3	0.4	0.4	0.3
	Treatment	1.4	2.5	3.2	3.4	3.5	3.5	2.2
Vitamin C (mg)	Control	18	40	54	62	68	65	51
	Treatment	94	167	219	236	239	233	153
Vitamin E (mg)	Control	3.4	7.2	10	12	13	12	9.4
	Treatment	20	35	45	49	50	48	32
β-carotene (mg)	Control	-	-	-	-	-	-	-
	Treatment	3.1	5.5	7.1	7.4	7.6	7.5	4.9
Zinc (mg)	Control	3.4	7.2	10	12	13	12	9.4
	Treatment	7.1	13	17	19	19	18	12
Selenium (μg)	Control	15	32	44	50	55	53	41
	Treatment	43	77	103	113	114	110	73
Module AOX units given	Control	-	-	-	-	-	-	-
	Treatment	0.57	1.01	1.29	1.36	1.39	1.38	0.89

Table 5 Surgery-induced response [difference between preoperative day (-1) and day after surgery (1) & difference between treatment and control groups over time, before start of intervention]

	Group	Day -1	Day 1	*P*-value[1]	Δ control and treatment group over time[2], *P*-value
Glutamine (μmol/L)	Control	557 (99)	434 (149)	0.021	0.888
	Treatment	596 (81)	457 (106)	0.007	
Vitamin C (μmol/L)	Control	53.5 (24.9)	22.3 (10.3)	0.003	0.379
	Treatment	68.6 (27.9)	29.4 (15.5)	0.007	
Vitamin E (μmol/L)	Control	27.4 (6.7)	11.0 (5.9)	0.003	0.458
	Treatment	29.1 (6.5)	10.9 (4.6)	0.005	
β-carotene (μmol/L)	Control	0.67 (0.78)	0.20 (0.23)	0.003	0.916
	Treatment	0.81 (1.09)	0.32 (0.45)	0.005	
Zinc (μmol/L)	Control	11.7 (2.1)	4.5 (2.1)	0.003	0.761
	Treatment	11.4 (2.6)	4.7 (2.0)	0.008	
Selenium (μmol/L)	Control	1.27 (0.28)	0.77 (0.23)	0.003	0.305
	Treatment	1.19 (0.25)	0.78 (0.21)	0.008	
MDA (μmol/L)	Control	10.29 (2.6)	12.61 (5.3)	0.091	0.035
	Treatment	10.32 (1.9)	9.35 (2.0)	0.285	
GSH-Px (U/g Hb)	Control	12.67 (3.55)	12.00 (2.54)	0.423	0.359
	Treatment	11.75 (2.08)	12.05 (1.63)	0.415	
Cysteine total (μmol/L)	Control	335 (46)	193 (40)	0.012	0.817
	Treatment	324 (39)	196 (56)	0.018	
WBC (E9/L)	Control	9 (2.7)	11.5 (5.2)	0.142	0.751
	Treatment	6.5 (1.6)	9.9 (3.4)	0.022	
IL-6 (pg/mL)	Control	10.7 (17.5)	261.7 (424.8)	0.003	1.000
	Treatment	3.0 (1.8)	337.2 (545.8)	0.005	

Data expressed as mean (standard deviation); MDA = malondialdehyde; GSH-Px = glutathione peroxidase; creat = creatinine; Hb = hemoglobin; WBC = white blood cell count; IL-6 = interleukin 6; [1]Wilcoxon Signed Rank Test; [2]Mann-Whitney *U* Test.

supplement for enteral nutrition on the indicators of oxidative stress and levels of antioxidants after major abdominal surgery. Major abdominal surgery induces oxidative stress that is associated with cellular dysfunction which may impair recovery. The rapid decrease in antioxidant levels on the first postoperative day indicates consumption to counter surgery-induced oxidative stress and is in accordance with earlier reports[32,33]. The levels of antioxidants could not be restored by the administration of Module AOX in the first five postoperative days, except for levels of selenium and glutamine (Table 5). These findings are in line with the results reported by Schroeder *et al*[33] who investigated the antioxidant enteral supplement Intestamin® (Fresenius Kabi) in similar major gastrointestinal tract surgical patients. They found that even at the higher dosage of glutamine, selenium, zinc, Vitamin C, Vitamin E and beta-carotene in Intestamin® compared to Module AOX, the antioxidant levels could

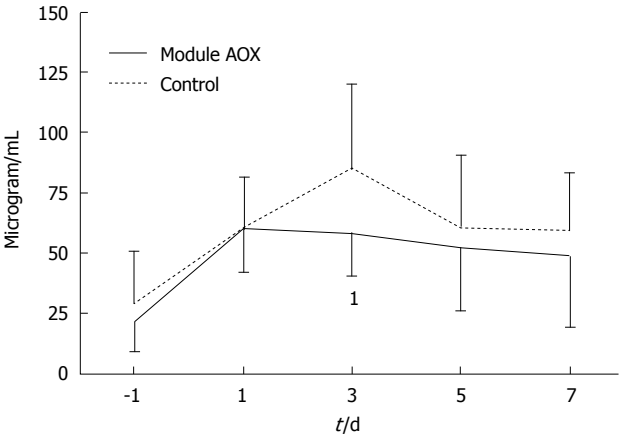

Figure 3 Development of LBP plasma concentration. Data represent means ± SD. [1]Indicates statistically significant difference between the control and treatment groups with respect to day 1.

not be raised to preoperative levels after five days of enteral nutrition. In contrast, in surgical critically ill patients, Intestamin® raised the plasma levels of glutamine, Vitamin C, Vitamin E and beta-carotene to normal levels at the third postoperative day[32]. A possible explanation for this discrepancy may be related to differences in the flow of antioxidants between cellular compartments in different patient populations and the capacity to absorb and metabolize supplemented antioxidants[34]. It should be noted that blood measurements only provide an approximation of the actual endogenous antioxidant defence status. Assessing antioxidant levels in other compartments or tissues is more difficult, but may better reflect the antioxidant defence status.

The blood concentrations of antioxidants present in Module AOX were significantly decreased on the first day after surgery. Only selenium and glutamine blood levels could be restored to preoperative levels, suggesting that the dosage of antioxidants was too low to influence blood levels. Theoretically, supplementing a combination of antioxidants that are depleted after surgery may have greater effects than supplying each antioxidant itself[10,35]. However, studies using some single antioxidant nutrients have shown important clinical results. This is especially true for glutamine which is considered a pharmaconutrient indispensable in critical illness. Glutamine appears beneficial in several patient groups, especially in those with burns, major trauma and after major surgery[36-39]. As an antioxidant, glutamine attenuates the inflammatory and oxidative stress response by enhancing plasma and tissue levels of glutathione[32]. The amount of glutamine supplementation recommended in a meta-analysis was 0.2-0.5 g per kg body weight which exceeds the amount of glutamine in Module AOX[39]. This may explain why there were no differences in glutamine levels between the control and Module AOX group after five days of nutrition.

As a single supplement, selenium is associated with decreased morbidity[16] and mortality[10] in critically ill patients. As a component of GSH-Px selenoenzymes,

Table 6 Postoperative response; 3, 5, 7 d after surgery

	Group	Day 3, *P*	Day 5, *P*	Day 7, *P*
Glutamine (μmol/L)	Control	494 (82)	566 (137)	624 (279)
		0.821	0.349	0.151
	Treatment	503 (91)	512 (108)	544 (83)
Vitamin C (μmol/L)	Control	18.3 (7.3)	23.1 (11.2)	32.2 (32.7)
		0.123	0.014	0.602
	Treatment	31.9 (13.5)	41.9 (13.9)	46.8 (16.6)
Vitamin E (μmol/L)	Control	17.6 (8.7)	21.9 (9.6)	20.6 (9.1)
		0.130	0.293	0.185
	Treatment	19.5 (4.7)	23.8 (8.7)	25.8 (11.6)
β-carotene (μmol/L)	Control	0.28 (0.31)	0.30 (0.28)	0.19 (0.23)
		0.458	0.301	0.087
	Treatment	0.34 (0.46)	0.39 (0.43)	0.45 (0.45)
Zinc (μmol/L)	Control	6.6 (2.9)	8.7 (3.4)	9.7 (3.6)
		0.704	0.426	0.280
	Treatment	6.9 (2.5)	8.5 (3.1)	10.3 (3.0)
Selenium (μmol/L)	Control	0.74 (0.26)	0.98 (0.25)	0.99 (0.25)
		0.123	0.002	0.006
	Treatment	0.89 (0.32)	1.15 (0.36)	1.29 (0.39)
MDA (μmol/L)	Control	11.55 (3.94)	11.71 (2.19)	10.69 (2.58)
		0.104	0.392	0.095
	Treatment	11.04 (4.21)	10.13 (1.98)	11.01 (2.73)
GSH-Px (U/g Hb)	Control	11.29 (1.39)	11.44 (1.76)	10.85 (1.51)
		0.648	0.945	0.165
	Treatment	11.84 (1.63)	11.83 (1.72)	11.78 (1.63)
Cysteine total (μmol/L)	Control	253 (49)	295 (50)	307 (84)
		0.836	0.278	0.419
	Treatment	251 (52)	308 (73)	320 (85)
WBC (E9/L)	Control	10.8 (2.1)	9.7 (2.9)	10.9 (4.1)
		0.595	0.447	0.893
	Treatment	8.2 (2.4)	6.3 (1.3)	10.0 (3.8)
IL-6 (pg/mL)	Control	91.6 (165)	25.1 (25.8)	33.7 (39)
		0.547	0.708	0.650
	Treatment	43.5 (37.6)	28.1 (27.6)	20.1 (16.3)

Data expressed as mean (standard deviation); MDA = malondialdehyde; GSH-Px = glutathione peroxidase; creat = creatinine; Hb = hemoglobin; GEE-analysis was used for statistical analysis; *P*, difference between the treatment and control group over time, corrected for baseline.

selenium inhibits nuclear factor kappa b (NFκB) which has a key role in the regulation of the expression of numerous cellular genes, particularly those involved in immune, inflammatory and stress responses. Therefore, by its effect on NFκB, selenium not only reduces inflammation, but also reduces oxidative stress and improves the defence mechanisms[40,41]. It is known that plasma GSH-Px is a sensitive marker of the response to antioxidant supplementation, especially selenium. Plasma GSH-Px declines in parallel with plasma selenium, while selenium supplementation restores the activity of the enzyme[16]. This is in contrast with the present study findings. Although the selenium plasma levels were raised after surgery in the treatment group, no difference in the plasma GSH-Px concentration was found. This suggests that the dosage of selenium given was insufficient to restore the activity of plasma GSH-Px.

Considering the above, one could argue that the dosages of glutamine and selenium are too low in Module AOX. However, increasing the dosages of antioxidants may be hazardous because at high intake some antioxidants may be toxic. In addition, as a consequence of their physical properties, some antioxidants also have pro-

oxidant effects. The capacity to scavenge free radicals is associated with the transformation of the scavenger into a free radical itself[16,34]. The possibility of an antioxidant acting as a pro-oxidant as well, could explain the unexpected increase in F2-isoprostane in the treatment group, although the dosages of the antioxidants used were low and established as safe. As for the other indicator of oxidative stress measured, MDA, no changes were observed in either of the groups. Similar to our findings, Preiser *et al*[42] also could not demonstrate any effect of an antioxidant-containing diet on levels of MDA in a randomised, double-blind, placebo-controlled study with critically ill patients during a seven day study period.

In the present study, malnutrition was an exclusion criterion, because it is an independent risk factor for the occurrence of postoperative infectious complications[43,44]. Kondrup *et al*[45] using the Nutritional Risk Screening (NRS 2002) examined how severity of illness and nutritional risk affected results in nutritional intervention studies. They found that better clinical effects were achieved in patients with a greater severity of illness and malnutrition[45]. It is possible that in the present study the patients who would have benefited most from antioxidant-enriched enteral nutrition, were excluded.

Lipopolysaccharide binding protein is an acute phase protein that is mainly synthesized by hepatocytes and its concentration in the circulation increases during inflammation[46]. At times of gut injury, such as during major surgery, LBP strongly modulates the response to endotoxins, which are present at the outer membrane of gram-negative bacteria (GNB). LBP-coated GNB are taken up mainly by monocytes and macrophages[46,47]. Endotoxins induce a receptor-mediated signalling cascade that leads to NF-κB activation and the transcription and subsequent release of cytokines and other proinflammatory mediators by monocytes and macrophages. Reactive oxygen species may be involved in the endotoxin-induced inflammatory response in two ways. Firstly, ROS may impair gut integrity by damaging the gut wall[48] inducing endotoxin translocation, and secondly ROS mediate endotoxin-induced NF-κB activation. It was demonstrated that neutralizing endotoxin, by recombinant bactericidal permeability increasing protein, lowered LBP levels in patients undergoing major liver resection[49]. In the Module AOX group, LBP levels decreased significantly compared to the control group which suggests that antioxidant supplementation modulates this acute phase response. This effect may be related to protection of gut integrity against ROS damage.

In major gastrointestinal tract surgical patients the oxidative stress parameters were not increased on the first postoperative day. Interestingly, in the antioxidant-supplemented group an increase in F2-isoprostane was observed during the first three postoperative days. This observation questions the use of antioxidants and further studies on the underlying mechanism are needed. A postoperative decrease in antioxidant levels occurred which could not be restored to the preoperative levels by Module AOX except for selenium levels. Larger multicenter studies are needed to further elucidate the effects of antioxidant-supplemented enteral nutrition in major gastrointestinal tract surgical patients. In such a trial, malnourished patients should also be included.

COMMENTS

Background

Major surgery and critical illness induce an immuno-inflammatory response, which is accompanied by the production of reactive oxygen species (ROS) at the site of injury. Reactive oxygen species can cause direct cellular injury by damaging lipids, proteins and DNA. In situations of major surgery and critical illness, a redistribution of antioxidants occurs to tissues or organs in need. This results in a depletion of antioxidant stores that may be deleterious when oxidative stress is prolonged. In these situations the supplementation of certain antioxidant amino acids (glutamine, cysteine) and antioxidant micronutrients (zinc, Vitamin C, Vitamin E, β-carotene, selenium) may improve outcome. The present study investigates the effect of antioxidant supplemented enteral feeding on circulating factors of the antioxidant defence system and markers of oxidative stress in patients after major upper gastrointestinal tract surgery.

Research frontiers

Theoretically, supplementing a combination of the antioxidants that are depleted after surgery may have greater effects than supplying each antioxidant itself. However, studies using some single antioxidant nutrients, like glutamine and selenium, have shown important clinical results, The effects of supplementation with combinations of antioxidants are still scarce, but the effects which are known are promising. Little is known about the effects of major abdominal surgery and antioxidant supplementation on the blood levels of parameters of antioxidant capacity and oxidative stress, which are important to know.

Innovations and breakthroughs

This study showed that oxidative stress parameters were not increased on the first postoperative day. A postoperative decrease in antioxidant levels occurred which could not be restored to the preoperative levels by Module AOX except for selenium levels. Interestingly, in the antioxidant-supplemented group, an increase in F2-isoprostane was observed during the first three postoperative days. This observation questions the use of antioxidants and further studies on the underlying mechanism are needed.

Applications

Larger multicenter studies are needed to further elucidate the effects of antioxidant-supplemented enteral nutrition in major gastrointestinal tract surgical patients. In such a trial, malnourished patients should also be included, since they might benefit most from such supplementation.

Peer review

This study investigates the effects of antioxidant enriched enteral nutrition on oxidative stress after major upper gastro-intestinal tract surgery. This study is well investigated and has some interesting findings.

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