Effect of a specific supplement enriched with n-3 polyunsaturated fatty acids on markers of inflammation, oxidative stress and metabolic status of ear, nose and throat cancer patients

M.M. ROCA-RODRÍGUEZ[1,2,4], J.M. GARCÍA-ALMEIDA[2], Y. LUPIAÑEZ-PÉREZ[3], J.M. RICO[3], M. TOLEDO[3], J. ALCAIDE-TORRES[1], F. CARDONA[1,4], J.A. MEDINA[3] and F.J. TINAHONES[1,2,4]

[1]Virgen de la Victoria Hospital Investigation Unit (IMABIS), Malaga; Departments of [2]Endocrinology and Nutrition, and [3]Clinical and Radiotherapeutic Oncology, Virgen de la Victoria Hospital, Malaga; [4]Biomedical Research Center of Physiopathology of Obesity and Nutrition (CIBER CB06/003), Carlos III Health Institute, Madrid, Spain

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Abstract. Malnutrition affects 40-50% of patients with ear, nose and throat (ENT) cancer. The aim of this study was to assess changes induced by a specific nutritional supplement enriched with n-3 polyunsaturated fatty acids, fiber and greater amounts of proteins and electrolytes, as compared with a standard nutritional supplement, on markers of inflammation, oxidative stress and metabolic status of ENT cancer patients undergoing radiotherapy (RT). Fourteen days after starting RT, 26 patients were randomly allocated to one of two groups, 13 supplemented with Prosure®, an oncologic formula enriched with n-3 polyunsaturated fatty acids, fiber and greater amounts of proteins and electrolytes (specific supplement), and 13 supplemented with Standard-Isosource® (standard supplement). Patients were evaluated before RT, and 14, 28 and 90 days after starting RT. The results showed that there were no significant differences between the groups, but greater changes were observed in the standard supplement group, such as a decline in body mass index (BMI), reductions in hematocrit, erythrocyte, eosinophil and albumin levels, and a rise in creatinine and urea levels. We concluded that metabolic, inflammatory and oxidative stress parameters were altered during RT, and began to normalize at the end of the study. Patients supplemented with Prosure showed an earlier normalization of these parameters, with more favorable changes in oxidative stress markers and a more balanced evolution, although the difference was not significant.

Correspondence to: Dr M.M. Roca-Rodríguez or Dr F.J. Tinahones, Virgen de la Victoria Hospital Investigation Unit (IMABIS), Campus Teatinos s/n, ES-29010 Malaga, Spain
E-mail: maroca80@gmail.com
E-mail: fjtinahones@hotmail.com

Abbreviations: BMI, body mass index; CDDP, cisplatin; CRP, C reactive protein; CV, coefficients of variation; ENT, ear, nose and throat; ESPEN, The European Society for Clinical Nutrition and Metabolism; GPx, glutathione peroxidase; GST, glutathione S-transferase; Gy, Gray; ICRU 50, International Commission on Radiation Units and Measurements; IL-1, interleukin-1; IL-6, interleukin-6; LPO, lipid peroxidation; MV, megavolts; NCI, National Cancer Institute; ROS, reactive oxygen species; RT, radiotherapy; SOD, superoxide dismutase; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor-α; TNM, tumor, nodes and metastasis classification

Key words: oxidative stress, inflammation, metabolic status, radiotherapy, n-3 polyunsaturated fatty acids

Introduction

Malnutrition affects 40-50% of patients with ear, nose and throat (ENT) cancer (1,2). Malnutrition in patients with head and neck cancer has been associated with increased rates of postoperative complications, a poor response to treatment and a greater percentage of tumor recurrence (3). Many of these patients require radiation therapy. The radiotherapy (RT) alters cellular homeostasis, modifying signal transduction pathways and the disposition to apoptosis (4). The acute toxicity of radiation is mediated by local inflammatory phenomena (5) and involves cytokines that are implicated in the cachexia-anorexia syndrome (6), such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α. Radiation also generates reactive oxygen species (ROS) by transferring energy to certain cellular components, followed by molecular changes, with damage to lipids, proteins and DNA. Cells have extensive protective mechanisms, such as enzymatic and non-enzymatic antioxidants that prevent or limit oxidative damage. The cellular superoxide anion generated by ionizing radiation can be rapidly dismutated to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide is then disposed of by the enzyme glutathione peroxidase (GPx) (7). Electrophiles are detoxified by conjugation reactions mediated by glutathione S-transferase (GST). ROS can be eliminated or inactivated *in vivo* by various endogenous molecules (e.g., uric acid, albumin) or by different exogenous antioxidants derived from the diet (8). The sum of endogenous and food-derived antioxidants represents the total antioxidant capacity (TAC) of the extracellular fluids.

After irradiation of ENT tissues, the development of mucositis, more or less severe, is constant (9). It affects the non-keratinized mucosa, soft palate, back of the tongue, floor of the mouth and oral cavity. Apart from the mucosal inflammation (10), there is a short fall in production of saliva due to the effect of radiation on the salivary glands (11). This results in great difficulty when chewing and swallowing food, with altered taste and local pain, leading to a decreased intake or even inability to eat. The end result is a serious trend in malnutrition among patients with ENT tumors undergoing local irradiation (12). These patients therefore require a nutritional supplement.

Considerable data support the beneficial effect of specific supplements and n-3 polyunsaturated fatty acids in the prevention and treatment of several chronic diseases, such as cardiovascular disease, inflammatory disease and cancer, generally related to the ability of n-3 fatty acids to induce apoptosis and cell cycle arrest in cancer cells (13,14). The intake of n-3 polyunsaturated fatty acids improves the quality of life and decreases weight loss in patients with advanced pancreatic cancer (15). In addition, n-3 polyunsaturated fatty acids can also reduce energy expenditure (16) and exert an anti-inflammatory action. However, not all studies agree on these positive effects (17).

The aim of the present study was to assess changes induced by two different nutritional supplements, one of them enriched with n-3 polyunsaturated fatty acids, fiber and greater amounts of proteins and electrolytes, on inflammation, oxidative stress and metabolic status of ENT cancer patients undergoing RT.

Materials and methods

The study included 26 patients with ENT cancer who required treatment with RT. The patients were evaluated following the clinical guidelines of the European Society for Clinical Nutrition and Metabolism (ESPEN) (18) before starting RT, and chemotherapy when it was necessary. Their theoretical energy and protein needs were calculated by Harris-Benedict equations x activity factor and 1.3-1.5 g of protein per kg of weight, respectively. The patients received supplements to fulfill their nutritional requirements. Fourteen days after starting RT, the 26 ENT cancer patients were allocated by simple random sampling (EudraCT 2008-000330-50) into two groups: the Prosure® group (n=13) or the Standard-Isosource® group (n=13).

The study was approved by the Ethics Committee of Virgen de la Victoria Hospital and was conducted in accordance with the Declaration of Helsinki. All the participants gave their signed consent after being fully informed of the goal and characteristics of the study.

Study design. This prospective three-month follow-up study involved two patterns of a randomly administered nutritional intervention given to ENT cancer patients receiving one month of RT, to compare a standard nutritional supplement with another supplement enriched with n-3 polyunsaturated fatty acids, fiber and greater amounts of proteins and electrolytes. The experimental group received a supplement of a specific enteral formula, Prosure®, which is enriched with n-3 polyunsaturated fatty acids and antioxidants (specific supplement) (Table I). The control group was also supplemented, but with a standard enteral formula, Standard Isosource®, which does not contain any extra n-3 polyunsaturated fatty acids (standard supplement) (Table I). The specific supplement group received a daily supply of 6 g of n-3 polyunsaturated fatty acids (3 Prosure® bricks); the rest of their requirements were supplied by standard supplements. The adequacy of these supplements was evaluated throughout the whole study by anthropometric, biochemical and hematologic variables, adjusting the rest of the supplements according to the individual needs. Fig. 1 shows the flow diagram of the progress through the phases of the randomized controlled trial. The patients were evaluated at baseline before the first dose of RT and 14 days after the first dose of RT (time 1), at which point they were randomized to either a nutritional supplement enriched with proteins or a nutritional supplement enriched with proteins and n-3 polyunsaturated fatty acids. The patients were then evaluated after the 28 days of RT (time 2), and 90 days after initiation of RT (time 3). The patients received their nutritional supplements from time 1 to time 3.

Table I. Nutrient content of the standard and specific supplements.

	Standard supplement	Specific supplement
Volume (ml)	250	240
kJ/U	1,102	1236
kJ/ml	4.19	5.03
mOsm/l	292	474
N (g)	1.6	2.6
Proteins (g)	10.2	16
Oligopeptides (%)	-	47.5
Carbohydrates (g)	35.5	44
Lipids (g)	8.7	6.1
EPA (g)		1.1
Ratio n6/n3	7/1	0.3/1
Fiber (g)	-	2.33
Sodium (mg)	125	360
Potassium (mg)	337	480

kJ, kilojoules; EPA, eicosapentaenoic acid.

The study included only patients in TNM stages 3 and 4. The exclusion criteria were: age <18 or >80 years, evidence of acute or chronic inflammatory disease, infectious disease, metastatic disease, prior radiation therapy in the head and neck area, edema and/or ascites, malabsorption, and a psychological or medical disorder that would prevent the signing of informed consent.

The RT technique was as follows: patients were treated with 6 MV photons from a linear accelerator equipped with a secondary multileaf collimator. All the patients underwent computed axial tomography simulation with a thermoplastic mask for immobilization, virtual simulation and application of a three-dimensional calculation algorithm. The volumes and dose specifications were designed according to recommendations of the International Commission on Radiation Units and Measurements (ICRU 50) (19). The patients underwent

Table II. Characteristics of ENT cancer patients.

	Standard supplement	Specific supplement	P-value
Gender (male/female)	10/3	11/2	0.619
Age (years)	61.08±10.68	61.00±14.66	0.724
BMI (kg/m^2)	27.53±3.97	25.27±4.63	0.311
Smoking status			
Non-smokers	4 (30.8)	3 (23.1)	0.705
Ex-smokers	7 (53.8)	7 (53.8)	1.000
Smokers	2 (15.4)	3 (23.1)	0.655
Tumor localization			
Cavum	1 (7.7)	3 (23.1)	0.317
Oral cavity	2 (15.4)	1 (7.7)	0.564
Oropharynx	2 (15.4)	2 (15.4)	1.000
Larynx	7 (53.8)	6 (46.2)	0.782
Others	1 (7.7)	1 (7.7)	1.000
Cancer stage			
III	6 (46.2)	4 (30.8)	0.527
IV	7 (53.8)	9 (69.2)	0.617
Karnofsky performance status			
70	1 (7.7)	2 (15.4)	0.564
80	4 (30.8)	2 (15.4)	0.414
90	4 (30.8)	4 (30.8)	1.000
100	4 (30.8)	5 (38.5)	0.739

Values are presented as mean ± SD or n (%). Differences in frequency distribution between groups were assessed by the Fisher test.

conventional RT (five fractions of 1.8-2 Gy per week) or accelerated schedules with concomitant overlay for 4 weeks.

Chemotherapy: the patients received 3 cycles of 100 mg/m^2 of cisplatin (CDDP). Patients deemed unfit for chemotherapy (23% of the patients) were offered treatment with cetuximab. No significant differences were found between patients with and without chemotherapy. The patients received 400 mg/m^2 of cetuximab (shock dose), after which a 250 mg/m^2 dose of cetuximab was administered concurrently weekly during the course of RT.

The patients completed a structured interview to obtain the following data: gender, age, medical history and tobacco consumption.

Toxicity and response assessments. The National Cancer Institute (NCI) Common Toxicity Criteria version 4.0 (20) were used for grading toxicities. Patients were monitored for skin, hematologic and mucosal toxicity throughout the follow-up period. The Karnofsky performance status was also examined.

Laboratory determinations. Fasting venous blood samples were drawn at four times: at baseline, 14 days after starting RT, immediately after ending RT, and 3 months after ending RT. Samples were collected in vacutainers with and without ethylenediaminetetraacetic acid and placed on ice. Samples were centrifuged at 4,000 rpm for 15 min at 4˚C. Plasma and serum were aliquotted and stored at -80˚C until analysis.

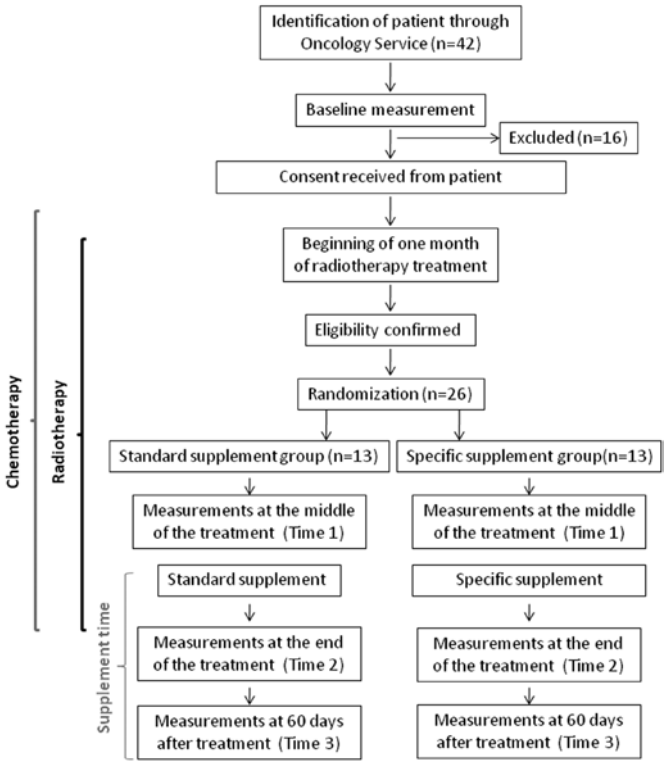

Figure 1. Flow diagram of the progress through the phases of the randomized controlled trial.

Table III. Distribution of hematological variables during the prospective study in patients with the standard supplement and the specific supplement.

	0	1	P-value	2	P-value	3	P-value	P-value
Standard supplement:								
n-3 acids from diet (g/d)	0.3±0.2	0.3±0.1		0.3±0.2		0.2±0.3		0.767
Basophils (x$10^3/\mu$l)	0.04±0.02	0.05±0.05		0.05±0.03		0.06±0.05[c]		0.958
Hematocrit (%)	40±6.6	38.1±6.9		36.3±6.2	0.019[a]	36.4±6.3	0.019[a]	0.050
Erythrocytes (x$10^6/\mu$l)	4.4±0.7	3.9±0.6	0.007[a]	3.8±0.6	0.003[a]	4.1±0.7		0.009
Hemoglobin (gr/dl)	13.4±2.1	12.4±1.9	0.021[a]	12.1±2	0.021[a]	12.1±2.4	0.010[a]	0.054
Leukocytes (x$10^3/\mu$l)	7.2±2.6	5.5±2.4		6.2±2.6		6.2±2.6		0.177
Lymphocytes (x$10^3/\mu$l)	1.5±0.6	0.7±0.3	0.001[a]	0.7±0.3	0.030[a]	0.9±0.4[c]	0.003[a]	<0.001
							0.039[b]	
Eosinophils (x$10^3/\mu$l)	0.2±0.1	0.1±0.1		0.1±0.04	0.049[a]	0.1±0.1		0.063
					0.014[b]			
Specific supplement:								
n-3 acids from diet								
and supplement (g/d)	0.299±0.363	0.295±0.164		1.006±0.264	0.003[a]	0.936±0.357	0.004[a]	0.043
					0.002[b]		0.003[b]	
Basophils (x$10^3/\mu$l)	0.1±0.04	0.04±0.02	0.002[a]	0.1±0.03		0.03±0.03[c]	0.002[a]	<0.001
Hematocrit (%)	38.7±5.2	35.9±3.5		35.3±3.5		34.9±5.2	0.033[a]	0.049
Erythrocytes (x$10^6/\mu$l)	4.2±0.6	3.9±0.5	0.041[a]	3.8±0.4	0.041[a]	3.8±0.5	0.034[a]	0.082
Hemoglobin (gr/dl)	12.9±1.8	11.9±1.2		11.5±1	0.028[a]	11.6±1.6	0.015[a]	0.015
Leukocytes (x$10^3/\mu$l)	8.6±3	6.9±3.7		6.1±2.7	0.015[a]	6.2±3.4		0.075
Lymphocytes (x$10^3/\mu$l)	1.6±0.6	0.9±0.8	0.034[a]	0.6±0.2	0.002[a]	0.8±0.4[c]	0.004[a]	<0.001
Eosinophils (x$10^3/\mu$l)	0.2±0.2	0.2±0.1		0.2±0.1		0.1±0.1		0.193

Values are presented as mean ± SD. P-value, statistical differences among different times in the same group of supplement according to the Friedman test. [a]Statistically significant differences between time 0 and the other times in the same group of supplement. [b]Statistically significant differences between time 1 and the other times in the same group of supplement. [c]Statistically significant differences between time 0 and the other times between the standard and the specific supplement group (P<0.05).

Serum biochemical parameters were measured in duplicate. Serum glucose (Randox Laboratories Ltd., Antrim, UK), uric acid (Dimension autoanalyzer; Dade Behring Inc. Deerfield, IL, USA), albumin, creatinine and urea (Siemens Healthcare Diagnostics Inc., Newark, DE, USA) were measured using standard enzymatic methods. C reactive protein (CRP) and IL-6 levels were measured using a human CRP Quantikine ELISA kit from R&D Systems (Abingdon, UK) and a human IL-6 Quantikine ELISA from R&D Systems according to the manufacturer's instructions. TAC, GPx, GST, and SOD activities were measured in plasma with commercial kits (Cayman Chemical, Ann Arbor, MI, USA). The intra- and inter-assay coefficients of variation (CV) of TAC were 3.4 and 3.0%, respectively. The intra- and inter-assay CV of GPx were 5.7 and 7.2%, respectively. The intra- and inter-assay CV of GST were 4.1 and 7.9%, respectively. The intra- and inter-assay CV of SOD were 3.2 and 3.7%, respectively. LPO levels were measured in serum using a commercial kit (Cayman Chemical).

Statistical analysis. The sample size was calculated for the main variable, oxidative stress: TAC [standard deviation (SD) =0.112 and mean difference =0.17] (21), with 95% power and an α error of 0.05. Sample size of each group: n=13.

The results are given as the mean ± SD. All clinical parameters are summarized by descriptive statistics. Information at each visit was compared by the Student's t-test for parametric variables and by the Friedman test for non-parametric variables. Differences in frequency distribution of qualitative variables between groups were assessed by the Fisher test. The Wilcoxon test for paired samples was used to compare oxidative stress, and biochemical and hematologic parameters between time 0 and the other times, and between time 1 and the others times (Bonferroni correction was applied). The Mann-Whitney U test was used to compare the studied variables between the two supplement groups. The Spearman correlation coefficient was calculated to estimate the linear correlations between variables. In all cases, the rejection level for a null hypothesis was α=0.05 for two tails. The statistical analysis was carried out with SPSS (version 15.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Table II shows the characteristics of the ENT cancer patients, with no significant differences between the groups.

The consumption of n-3 polyunsaturated fatty acids in each group at different times is shown in Table III, with similar

Table IV. Distribution of the Karnofsky and toxicity values during the prospective study in both groups of patients.

A, Karnofsky

	Standard supplement (n=13)					Specific supplement (n=13)					
	60	70	80	90	100	60	70	80	90	100	P-value
Time 0	0	1	4	4	4	0	2	2	4	5	0.774
Time 1	0	4	1	6	2	0	3	3	4	3	0.627
Time 2	1	4	1	5	2	2	2	4	3	2	0.509
Time 3	0	2	3	5	3	1	1	3	4	4	0.811

B, Hematologic toxicity

	Standard supplement (n=13)			Specific supplement (n=13)			
	0	1	2	0	1	2	P-value
Time 1	7	4	2	10	2	1	0.465
Time 2	11	1	1	10	2	1	0.827
Time 3	13	0	0	13	0	0	-

C, Mucosal toxicity

	Standard supplement (n=13)					Specific supplement (n=13)					
	0	1	2	3	4	0	1	2	3	4	P-value
Time 1	0	2	9	2	0	0	4	5	4	0	0.290
Time 2	0	0	5	8	0	2	0	4	7	0	0.337
Time 3	5	7	1	0	0	4	5	2	1	1	0.596

D, Skin toxicity

	Standard supplement (n=13)				Specific supplement (n=13)				
	0	1	2	3	0	1	2	3	P-value
Time 1	1	3	7	2	1	4	6	2	0.974
Time 2	2	2	5	4	4	4	2	3	0.430
Time 3	11	2	0	0	10	2	0	1	0.592

levels at times 0 and 1. Significant differences between the groups were observed after the patients were randomized to a standard or specific nutritional supplement, at times 2 and 3 (P=0.027 and 0.023, respectively).

Furthermore, Table III also summarizes the differences in hematological variables. Both groups experienced a parallel evolution; however, variables showed more significant differences in the standard supplement patients throughout the study. We observed that the standard supplement patients experienced significant changes in hematocrit, erythrocytes and eosinophils during the study. On the other hand, significant changes in basophils, hematocrit, hemoglobin and lymphocytes were found in the specific supplement group. When comparing the two groups, the basophil and lymphocyte levels were significantly lower between times 0 and 3 in the specific supplement group (P=0.002 and 0.035, respectively).

The Karnofsky and toxicity values remained similar in both groups of patients throughout the study. During RT, the Karnofsky values decreased and toxicity markers increased. Two months after the end of RT, the Karnofsky and toxicity markers improved (Table IV).

In both treatment groups, the body mass index (BMI) decreased significantly at first and recovered later with no significant differences, although a smaller decline in BMI was observed in the specific supplement patients (Table V).

Table V. Distribution of BMI and biochemical variables during the prospective study in patients with the standard supplement and the specific supplement.

	0	1	P-value	2	P-value	3	P-value	P-value
Standard supplement: n-3 acids from diet								
BMI (kg/m^2)	27.5±3.9	26.5±4.1	0.010[a]	25.79±4.40	0.003[a] 0.010[b]	26.09±4.72		0.004
Glucose (mmol/l)	7.9±4	8.4±4.7		8.31±5.29		5.85±1.84	0.017[b]	0.095
Albumin (g/dl)	3.6±0.4	3.3±0.4		3.230±0.673		3.400±0.710		0.177
Uric acid (mmol/l)	0.3±0.1	0.3±0.1		0.3±0.2		0.3±0.1[c]	0.005[a] 0.016[b]	0.081
Creatinine (μmol/l)	77.1±17.5	88.4±25.5		110.8±62.2[d]	0.008[a]	85.3±16.8		0.088
Urea (mmol/l)	5.7±2.5	8.5±5.1	0.008[a]	12.5±10.4	0.005[a]	6.9±3.5	0.044[a] 0.028[b]	0.001
Specific supplement: n-3 acids from diet and supplement								
BMI (kg/m^2)	25.3±4.6	24.7±4.5		24.±4.4	0.011[a] 0.023[b]	24.4±4.4		0.035
Glucose (mmol/l)	5.9±2.	6.7±1.9		6.5±3.5		5.6±2.4		0.198
Albumin (g/dl)	3.8±0.3	3.6±0.4	0.048[a]	3.4±0.2	0.012[a]	3.8±0.5	0.025[b]	0.013
Uric acid (mmol/l)	0.3±0.1	0.3±0.1		0.2±0.1		0.2±0.1c		0.281
Creatinine (μmol/l)	66.7±19.5	78.9±25.6	0.036[a]	72.4±18[d]		74.1±17.8		0.261
Urea (mmol/l)	5.8±2.1	7.9±4.2	0.027[a]	7.7±3.3	0.023[a]	8.5±4.9		0.020

Values are presented as mean ± SD. P-value, statistical differences among different times in the same group of supplement according to the Friedman test. [a]Statistically significant differences between time 0 and the other times in the same group of supplement. [b]Statistically significant differences between time 1 and the other times in the same group of supplement. [c]Statistically significant differences between time 0 and the other times between the standard and the specific supplement group (P<0.05). [d]Statistically significant differences between time 1 and the other times between the standard and the specific supplement group (P<0.05).

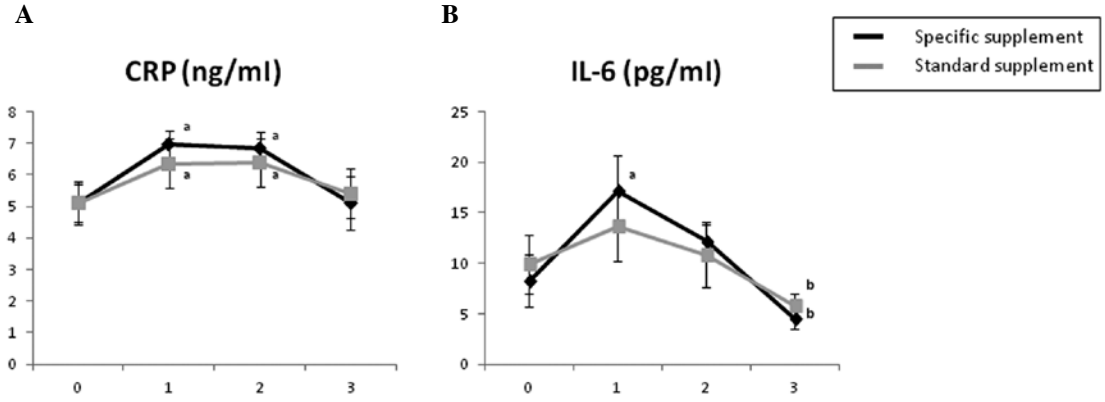

Figure 2. Distribution of inflammatory parameters (A and B), during the prospective study, in patients with the standard supplement (grey) and the specific supplement (black). The Wilcoxon test for paired samples was used to compare oxidative stress parameters between time 0 and the other times, and between time 1 and the others times (Bonferroni correction was applied). [a]Statistically significant differences between time 0 and the other times. [b]Statistically significant differences between time 1 and the other times (P≤0.05).

Concerning the biochemical variables (Table V), both groups experienced significant changes in levels of urea and, unexpectedly, in levels of albumin in the specific supplement group. Comparing both groups, we found that uric acid levels behaved differently according to the group. Patients receiving the standard supplement had significantly higher levels of uric acid whereas the patients receiving the enriched supplement had significantly lower levels of uric acid at time 3. Creatinine levels were also significantly increased at time 2 in the patients who received the standard supplement.

Regarding inflammatory variables, levels of CRP and IL-6 changed significantly in the specific supplement group (P=0.028 and 0.025, respectively) during the study. CRP levels were higher during RT at times 1 and 2 in both groups compared with time 0 (Fig. 2A) whereas levels of IL-6 rose at time 1 in both groups, more so in the specific supple-

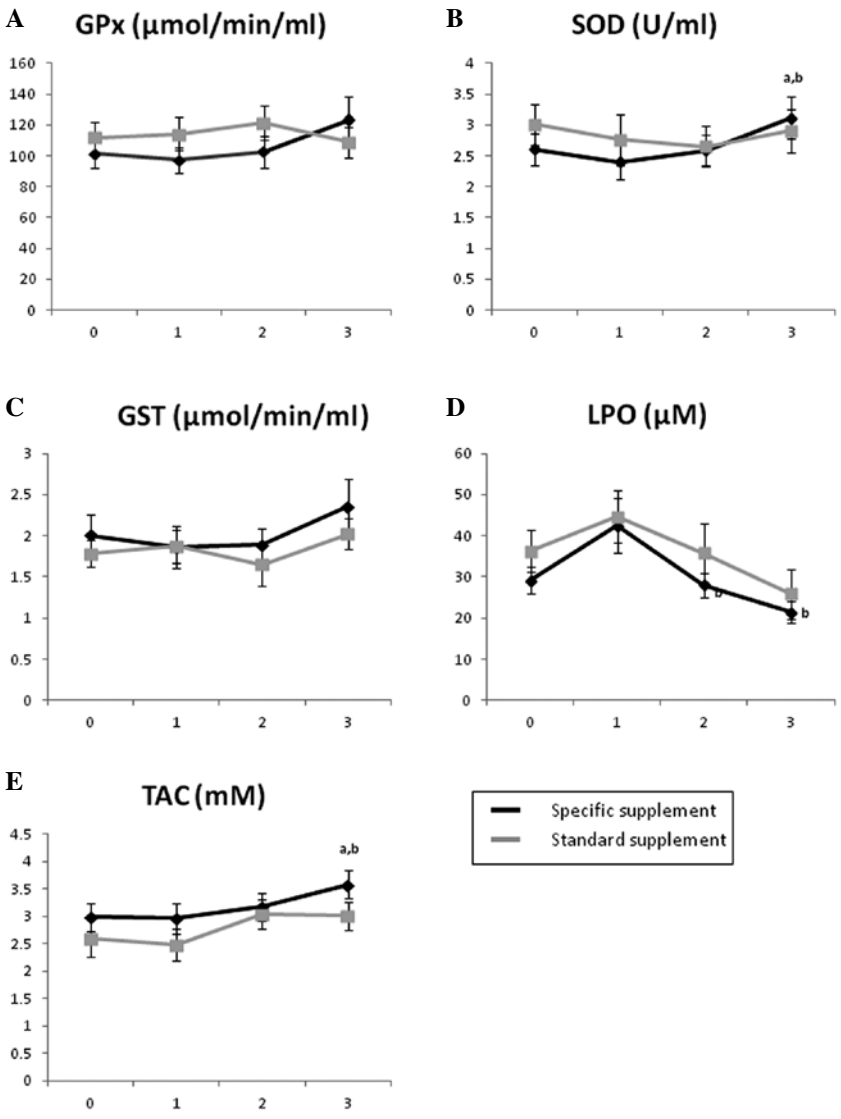

Figure 3. Distribution of oxidative stress parameters (A-E), during the prospective study, in patients with the standard supplement (grey) and the specific supplement (black). The Wilcoxon test for paired samples was used to compare oxidative stress parameters between time 0 and the other times, and between time 1 and the other times (Bonferroni correction was applied). [a]Statistically significant differences between time 0 and the other times. [b]Statistically significant differences between time 1 and the other times (P≤0.05).

ment group, but then fell in both groups at the other times (Fig. 2B).

The oxidants and antioxidant variables, LPO, GPx, SOD and TAC activity, changed significantly during the study in the specific group but not in the standard group (P=0.036, 0.029, 0.045 and 0.036, respectively). No significant differences were found for GST. In both groups, TAC and SOD activity increased significantly at the end of the study (time 3), and levels of LPO decreased significantly at the end of RT and at the end of the study (times 2 and 3) in patients who received the specific supplement (Fig. 3).

Significant negative correlations were observed between the Karnofsky values and inflammation parameters. The Karnofsky correlated with IL-6 levels at time 0 (Rs=-0.420, P<0.05, respectively), with CRP and IL-6 levels at time 1 (Rs=-0.470, P<0.05 and Rs=-0.394, P<0.05, respectively), and with CRP and IL-6 levels at time 3 (Rs=-0.684, P<0.001 and Rs=-0.631, P<0.005, respectively). Hematologic toxicity correlated negatively with TAC (Rs=-0.536, P<0.005).

Discussion

Cancer patients suffer metabolic and analytical abnormalities due to the disease and its treatments. RT often results in hematological and immune dysplasia as hematological stem cells are damaged during the procedures and committed hematologic and immune cells are then depleted (21,22). During the study, the hematologic variables decreased in both groups. These changes were more significant in the standard supplement patients. It thus seems that the hematologic variables of the patients taking the standard supplement were more affected by the treatment.

Cancer-associated weight loss is due in part to the lower food intake, particularly in ENT cancer patients for whom food intake frequently presents a real challenge. In addition, RT results in such conditions as mucositis, dysphagia or xerostomia, which promote weight loss; in addition, these symptoms all worsen if chemoradiation therapy (chemoRT) is needed. In our study, BMI and Karnofsky values decreased

and toxicity markers increased during RT. Two months later, these three variables improved. Some studies have evaluated weight loss in head and neck cancer patients and found that reductions in intake and weight were significantly greater in chemoRT than in RT patients (23).

We observed that albumin levels decreased at time 2, significantly so in the specific supplement group, and tended to normalize, almost reaching baseline levels at time 3. This is in agreement with previous studies which reported that RT decreased albumin levels (24) and n-3 polyunsaturated fatty acids increased serum albumin (25). However, we observed no significant benefit with the specific supplement regarding albumin levels.

Uric acid production is greatly increased after treatment with chemotherapy or RT as a result of increased purine breakdown. After RT, the two groups of patients showed a different behavior. The standard supplement patients showed a significant increase in levels of uric acid at the end of the study, whilst the specific supplement patients showed a significant decrease. Several studies have reported that n-3 polyunsaturated fatty acids reduce levels of uric acid (26,27). One explanation for this is that n-3 polyunsaturated fatty acids seem to reduce the end products of xanthine oxidase activity. The levels of urea increased significantly in both groups, more so than in the specific supplement patients, during and after the RT. The rate of urea production is an indicator of net protein catabolism, which increases significantly during periods of severe metabolic stress (28). In addition, urea production (28) and levels of uric acid (29) increase with the advancing tumor state. Thus, the patients receiving the specific supplement seem to have experienced less metabolic stress.

The inflammatory parameters CRP and IL-6 correlated negatively with the Karnofsky performance status. During the RT, inflammatory parameters were increased in both groups of patients. Exposure to radiation initiates a programmed molecular and cellular response to promote tissue repair, which includes the upregulation of proinflammatory cytokines (30). It is well-known that inflammation is a critical component of tumor progression and that nutritional supplements seem to improve inflammatory parameters, with contradictory findings in the literature between the specific and standard supplements (31). After 14 days of nutritional supplements (time 2), IL-6 levels began to decrease, and, at the end of the study, the IL-6 levels were significantly decreased and the CRP levels were normalized. Both CRP (29,32-34) and IL-6 (35-38) have a prognostic value in head and neck tumors, through direct relations with tumor state, tumor progression, metastasis and tumor response to treatment, and therefore in disease-free survival and overall survival. Some studies observed that blocking IL-6 signaling attenuated aggressive tumor behavior and sensitized the cells to treatments (39). Thus, any intervention which decreases levels of IL-6 (40) and/or CRP would have beneficial effects on tumor prognosis. In addition, CRP may be an objective and sensitive marker of radiation-induced mucositis (41) and an independent predictor of weight loss (23).

On the one hand, ENT cancer has a strong link to oxidative damage and stress, such as tobacco and alcohol, both sources of massive quantities of ROS, and, on the other hand, ionizing radiation causes severe cellular damage and stress, both directly by energetic disruption of DNA integrity and indirectly as a result of the formation of intracellular free radicals (42). Thus, parameters of oxidative stress could represent a useful tool for predicting the prognosis of head and neck cancer (43). In the present study we, as others (31,44), observed that subjects receiving RT, whichever nutritional supplement they were taking, displayed parameters of oxidative stress that were altered by the RT and that the TAC correlated negatively with toxicity. At the end of the study, oxidative stress parameters showed a trend towards normalization, although these alterations in the patients who received the specific supplement normalized to baseline levels earlier than in the group receiving the standard supplement. The antioxidant parameters and LPO levels also improved significantly two months after ending the RT. There have been conflicting reports on the effects of n-3 polyunsaturated fatty acid supplementation on oxidant/antioxidant status in humans (45,46). Several studies have observed significant associations in head and neck cancer between SOD and GPx activity and better disease-specific survival and postoperative RT (47), between manganese SOD and tumor migration and invasion (48), and between the loss of GPx expression and tumor chemoresistance (49), all important prognostic factors for survival. There is also a deranged antioxidant defense system due to significantly low levels of TAC (50) and higher levels of nitric oxide and GST in these cancer patients, with a significant increase after RT (51). By contrast, we found no significant differences in GST. Our results suggest that the supplement enriched with n-3 polyunsaturated fatty acids prevents patients from experiencing abrupt changes in oxidative stress parameters during treatment. It has been postulated that n-3 polyunsaturated fatty acids may reduce oxidative stress by allowing a reduction in the disease inflammatory activity, thus decreasing the production of free radicals, or by acting as a free radical scavenger (52). Moreover, docosahexaenoic acid has a protective effect against H_2O_2-induced oxidative stress in human lymphocytes (53).

We recognize several limitations in this study, such as the small patient population sample, the heterogeneous and contradictory publications evaluating the immunonutrition support with which to compare our results, and the dietary intervention itself, which was more than just n-3 polyunsaturated fatty acids. The contradictory data could be explained by the differences in nutritional support formulas. We tried to reduce this limitation by providing only 3 Prosure bricks per day in the specific supplement group whereas the rest of their requirements were supplied by standard supplements. In addition, pathophysiological changes in cancer patients make it difficult to establish causal relationships. Nevertheless, it appears that a formula containing amino acids, n-3 fatty acids and ribonucleic acids could modulate the inflammatory and antioxidant response.

In conclusion, cancer and its treatment, including chemotherapy and RT, produce metabolic stress and alterations in parameters of inflammation and oxidative stress. Nutritional supplements seem to improve these alterations, leading to a better health status in these ENT cancer patients. The nutritional intervention appears to influence the oxidative and inflammation state. The specific supplement does not produce significantly long-lasting differences in markers of inflammation, but it does produce more favorable changes in markers of oxidative stress, less metabolic stress and, in general, a

more balanced evolution with respect to the standard diet. These changes do not produce changes in quality of life over the short term. Finally, a long-term follow-up could provide additional insights into the effect of nutritional supplements on quality of life.

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