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Supplementation with antioxidant micronutrients and chemotherapy-induced toxicity in cancer patients treated with cisplatin-based chemotherapy: a randomised, double-blind, placebo-controlled study ☆

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Abstract

Cisplatin-induced toxicities are mainly caused by the formation of free radicals, leading to oxidative organ damage. Plasma concentrations of antioxidants decrease significantly during cisplatin chemotherapy for cancer. Forty-eight cancer patients treated with cisplatin-based chemotherapy were randomised in a double-blind manner to receive either supplementation with vitamin C, vitamin E and selenium dissolved in a beverage or to receive a placebo beverage. Primary outcome measures were the amount of nephrotoxicity and ototoxicity induced by cisplatin. No significant differences were found between the two study groups with respect to these primary outcome measures. However, patients who achieved the highest plasma concentrations of the three antioxidant micronutrients had significantly less loss of high-tone hearing. In addition, significant correlations were found between the reduced/oxidised vitamin C ratio and malondialdehyde (MDA), markers of oxidative stress, and cisplatin-induced ototoxicity and nephrotoxicity. The lack of protection against cisplatin-induced toxicities in patients in the intervention arm may be related to poor compliance and/or inadequate supplementation. Supplementation with a higher dose (intensity) and in combination with other antioxidants should be investigated further.

Keywords: Antineoplastic agents; Antioxidants; Ascorbic acid; Cisplatin; Free radicals; Neoplasms; Oxidative stress; Selenium; Vitamin E

1. Introduction

Chemotherapy-related toxicity is a major concern in the treatment of patients with solid tumours, particularly in those patients who are cured or achieve prolonged survival. Besides acute toxicity occurring immediately following the administration of cytostatic agents, e.g., nausea, alopecia, oral mucositis [1] and bone marrow depression, long-term side-effects can reduce the quality of life of these patients. Cisplatin is currently one of the most important cytostatic agents in the treatment of a wide range of solid tumours. It is the mainstay in the potentially curative combination chemotherapy of patients with disseminated testicular and ovarian cancer or in neo-adjuvant treatments for osteosarcoma. It is also frequently used in the palliative treatment of metastatic gastrointestinal, urogenital, lung and head and neck cancers. Nephrotoxicity, loss of high-tone hearing and peripheral neuropathy are the most important, in part irreversible, long-term side-effects of cisplatin [2].

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Formation of free radicals, leading to oxidative stress, has been shown to be one of the main pathogenetic mechanisms of these toxicities and side-effects of other cytostatics, such as doxorubicin cardiomyopathy and bleomycin pulmonary damage [3]. Administration of antioxidant agents in animals, but also in clinical studies, has been proven to ameliorate or prevent some of these side effects. However, specific toxicities of the investigated substances sometimes led to dose reduction, in the antitumour agents, decreased response rates or even an enhancement of the toxicity which was meant to be reduced [4–6].

Recently, we (and others) have demonstrated that plasma concentrations of various antioxidants decreased significantly during cisplatin-based chemotherapy in cancer patients [7–9]. The decreased levels of the physiological antioxidant defense mechanism may lead to increased oxidative stress and free radical-mediated organ damage. Vitamin C, vitamin E and glutathione peroxidase (of which selenium is an essential constituent) are some of the most important natural antioxidants, which are synergistically able to interfere with the harmful reactions initiated by free radicals [3]. To investigate the role of oral supplementation of vitamin C, vitamin E and selenium in the prevention of cisplatin-induced toxicity, we have performed a randomised, placebo-controlled, double-blind study in patients with solid tumours treated with cisplatin-containing chemotherapy. Patients were randomised to receive either supplementation with vitamin C, E and selenium or placebo.

2. Patients and methods

Between February 1995 and August 1997, patients at the department of Clinical Oncology of the Leiden University Medical Center eligible for cisplatin single agent or combination chemotherapy were randomised in a double-blind manner into two groups who received either supplementation with vitamin C, E and selenium or placebo. This combination of vitamin C, E and selenium will be further referred to as ''antioxidant micronutrients''. Stratification was made according to the planned cisplatin dose intensity: ''high'' ($\geqslant 70$) or ''low'' (<70 mg/m^2/day). Exclusion criteria for enrolment were: (1) Prior chemotherapy. (2) Supplementation with vitamin C, E or selenium during the 30 days before inclusion or treatment with other antioxidative during chemotherapy. (3) Calculated creatinine clearance <1.33 ml/s (<80 ml/min). (4) Karnofsky performance status $<50\%$. Baseline audiometrical findings were not part of the patients' selection criteria. The study was approved by the ethics committee and informed consent was obtained from all of the patients.

Fifty patients were included in the study (Fig. 1). Two patients, randomised into the placebo group, were excluded from the analysis because of early death due to rapid tumour progression ($n = 1$) and a cerebral ischeamic stroke [10] ($n = 1$). The remaining 48 patients suffered from various malignant tumours, i.e. testicular cancer ($n = 16$), (osteo) sarcoma ($n = 13$), gastrointestinal cancer ($n = 6$), urogenital cancer ($n = 5$), head and neck cancer ($n = 5$) and melanoma ($n = 3$).

Both groups received 100 ml of a milky beverage with a vanilla flavour containing 3.42 g protein, 7.92 g carbohydrate and 0.05 g fat twice a day, beginning 7 days before the onset of chemotherapy until 3 weeks after cessation of therapy. Twenty-five patients were randomised to receive these beverages in which 1000 mg vitamin C (as L-ascorbic acid), 400 mg vitamin E (as dl-α-tocopherol-acetate) and 100 μg selenium (as sodium selenite) were dissolved (''supplementation'' or ''intervention'' group). Twenty-three patients received the same beverages without these antioxidants (''placebo group''). Compliance to supplementation was recorded by the patients in checklists filled in every day.

The primary outcome measures were acute (i.e. during chemotherapy) and long-term (i.e. 2 and 12 months after completion of chemotherapy) nephrotoxicity and ototoxicity induced by cisplatin (combination) chemotherapy. Nephrotoxicity was measured by the decrease in the calculated creatinine clearance and ototoxicity by the loss of high-tone hearing. The secondary outcome measures were the occurrence of chemotherapy-induced anaemia, leucocytopenia and thrombocytopenia. The efficacy of supplementation was measured by the plasma concentrations of vitamin C, vitamin E and selenium.

Patients received cycles of intravenously (i.v.) administered chemotherapy, each cycle consisting of 1–5 days of cytostatic drug infusions, repeated every 21 days. Patients received 3–4 l/day of 0.9% sodium chloride and 5% glucose i.v. to reduce cisplatin-mediated nephrotoxicity. Known nephrotoxic or ototoxic medication, e.g. aminoglycosides and amphotericin, were not administered to patients. In case of water overload, the first-line diuretic was mannitol. Only a few patients received furosemide 10 mg i.v. once or twice during the whole course of treatment in cases of water retention.

2.1. Blood sampling

Blood specimens were drawn one day prior to initiation of the first, second and fourth chemotherapy courses (''day 1''), within 24 h after cessation of the chemotherapy infusions on the last day of hospital admissions (usually on day 2–5, depending on the chemotherapy scheme) for the first and fourth chemotherapy course, at the ''nadir days'' of the 1st and 4th chemotherapy course in the outpatient department (usually between days 8 and 15), and 2 and 12 months

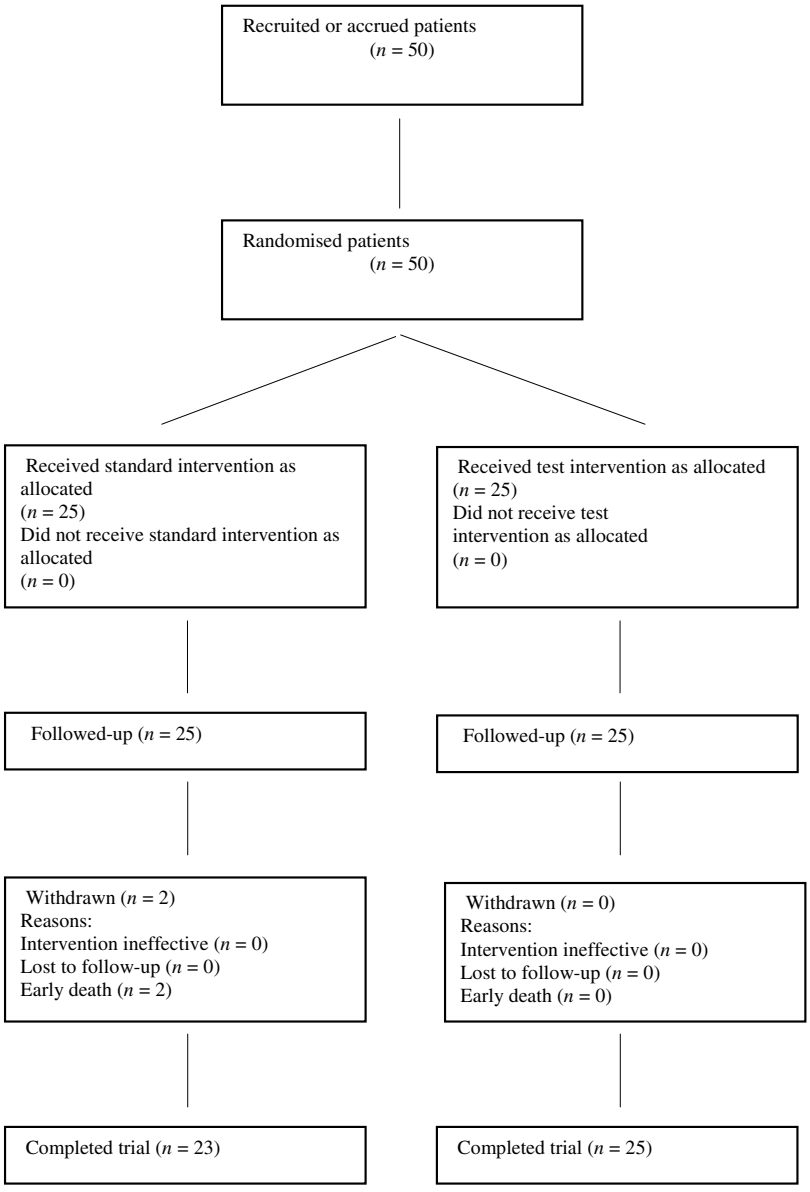

Fig. 1. Flow chart of the progress of patients through the trial.

after completion of chemotherapy. Collection, processing and storage of blood and urine samples were performed as previously described in [7].

2.2. Analytical methods

Total vitamin C, reduced and oxidised vitamin C [11,12], vitamin E [13] and malondialdehyde (MDA) [14] were measured by high performance liquid chromatography (HPLC). The ratio of reduced and oxidised vitamin C (i.e. ascorbic acid and dehydroascorbic acid, respectively) and plasma concentrations of MDA were used as markers of oxidative stress. A relative increase in oxidised vitamin C (and thus a decrease in the reduced/oxidised vitamin C ratio) and increase of MDA is indicative of oxidative stress. Concentrations of cholesterol, triglycerides, magnesium (normal value 0.65–0.98 mmol/l) and creatinine were determined on a Synchron CX4CE clinical chemical analyzer (Beckman Instruments Inc., Brea, California, USA). Plasma selenium was determined by atomic absorption spectroscopy. The lipid standardized vitamin E plasma concentration (vitamin E/lipids ratio), a parameter for the functional activity of vitamin E [15], was calculated as the plasma vitamin E concentration divided by the sum of the plasma concentrations of cholesterol and triglycerides.

Patients' individual plasma concentrations of vitamin C, E and selenium on day 1 of the first chemotherapy cycle were assigned an arbitrary value of 2 or 1. Two, indicating that their individual plasma concentration of

vitamin C, E or selenium was more than the median value of the whole group. One, indicating that their individual value was equal to or less than the median value of the whole group. Thereafter, the obtained values were added resulting in an ''overall micronutrient antioxidant score'' ranging from 3 to 6 for each patient.

2.3. Renal and hearing function

Creatinine clearance (24 h urinary creatinine/plasma creatinine), reflecting the glomerular filtration rate, was used as a parameter for renal function. Plasma magnesium concentrations were used as indicators of renal tubular function, as renal tubular magnesium wasting often occurs in patients treated with cisplatin. Prior to chemotherapy and just before each subsequent chemotherapy cycle, audiological examination was performed as previously described in [2,7].

2.4. Statistical analysis

Osanto et al. [2] found that the creatinine clearance decreased from 2.12 ± 0.53 to 1.68 ± 0.38 ml/s in cisplatin-treated patients. This is an effect-size of approximately 0.9 ((127–101)/28, where 28 is the pooled standard deviation). Auditive threshold at 8000 Hz increased from 11 to 21 dB with a similar effect-size. We expected that this cisplatin-associated damage could be fully abolished by vitamin supplementation. Accordingly, we powered the study to have 80% power for the expectation of effect-sizes of 0.8 or more (0.05 two-sided significance level), and therefore included 24 patients per treatment group.

The statistical analysis approach was based on an intention-to-treat analysis. Paired Student's *t*-test was used to compare the values of parameters of individual patients during one particular cycle and the values on day 1 of two distinctive cycles. Independent Student's *t*-test was used to compare mean values of parameters between both study groups. For comparisons between both study groups with respect to categorical parameters, Mann–Whitney *U* test or the χ^2 test was performed, where appropriate. Bivariate Pearson's correlation coefficients were used in order to investigate the relationships between the various parameters concerning antioxidants and oxidative stress and those parameters indicating chemotherapy-induced toxicity. Differences between groups and correlation coefficients were considered significant if $P < 0.05$.

3. Results

Forty-eight patients, 23 in the placebo arm and 25 in the active arm, were analysed. Patients' characteristics are shown in Table 1. There were no significant differences between the groups with respect to gender, age and anthropometric parameters. Although the planned cisplatin dose (mg/m^2) per day was equally distributed over the two groups, more patients in the intervention

Table 1
Patients characteristics

Parameters	Placebo ($n = 23$)	Supplementation ($n = 25$)	*P* value[a]
Male/female	20/3	21/4	NS
Age[b,c](years) (median; range)	42 ± 16 (47; 16–66)	44 ± 16 (46; 24–69)	NS
Body weight[b,c] (kg)	69.8 ± 13.3	77.2 ± 13.7	NS
Body mass index[b,c] (body weight/body length^2; kg/m^2)	22.5 ± 4.4	24.0 ± 3.6	NS
Planned cisplatin dose intensity/day (mg/m^2)			
Mean ± SD	69 ± 29	67 ± 35	NS
20	4	7	
50–60	7	4	
70–80	4	3	
100	8	11	
Planned cisplatin dose intensity/cycle (mg/m^2)			
Mean ± SD	89 ± 16	97 ± 8	0.04
60	4	0	
70	1	1	
75	1	1	
80	2	1	
100	15	22	
Total dose of cisplatin administered (mg/m^2); mean ± SD)	371 ± 139	339 ± 125	NS

SD; standard deviation.
[a] *P*-value for comparison between values in both randomisation groups (independent-samples Student's *t*-test or χ^2 test were used as appropriate); NS; non-significant.
[b] At the start of chemotherapy.
[c] Mean ± SD.

group received the highest planned cisplatin dose of 100 mg/m^2 per cycle compared with the placebo group in their first chemotherapy course (Table 1). Dose modifications applied during subsequent chemotherapy cycles resulted in equal mean total doses of cisplatin administered to patients of both groups.Tumour types were equally distributed over the two arms. Observed tumour responses for the patients, including 15 patients with complete remission (CR), 7 in partial remission (PR) and 4 patients with stable disease (SD) were equally divided in both groups. The remaining patients had progressive disease ($n = 11$) or were not evaluable for overall response as they had received neo-adjuvant chemotherapy prior to surgery for a primary osteosarcoma ($n = 11$). In the intervention group, 9 patients had a CR and 2 patients a PR (overall response 44%). In the placebo group, 6 patients had a CR and 5 had a PR (overall response 48%). These percentages of clinical remissions were as expected for the type and stage of the various tumours.

Data about the plasma concentrations of antioxidant micronutrients and MDA before (day −7) and after seven days of supplementation (day 1) are shown in Table 2. Concentrations of the antioxidant micronutrients vitamin C, E and selenium and MDA did not differ between the two groups of patients at the time of enrolment (Table 2, day −7). At day 1, one week after enrolment, levels of vitamin C, E and selenium in all supplemented patients had increased to significantly higher levels compared with their levels before the start of the intervention (day −7) and the levels of the placebo group at day 1 (Table 2, all P values <0.001). MDA concentrations did not differ significantly between both groups before and after supplementation (Table 2).

Following the first chemotherapy infusions, plasma levels of vitamin C and E, as well as vitamin E/lipids ratios, significantly decreased at the nadir day of cycle 1 in both groups of patients (Table 3; all P values, were <0.001). The absolute fall in plasma antioxidant levels was greater in the supplementation group than in the placebo group. At the nadir day of cycle 1 and during further chemotherapy, plasma levels of vitamin C and E of patients in the supplementation group remained significantly higher than those in the placebo group (Table 3; $0.001 < P < 0.008$). Selenium levels in the placebo group decreased significantly after the first chemotherapy cycle ($P = 0.028$) and remained at that lower level during the subsequent chemotherapy cycles (Table 3). Plasma selenium levels in the supplementation group did not change significantly during the whole chemotherapy treatment period.

Two and 12 months after completion of chemotherapy, plasma micronutrient antioxidants concentrations did not differ between the two groups (data not shown).

In the supplementation group, after a slight decrease in plasma MDA levels during the 7 supplementation days prior to the initiation of chemotherapy (Table 2), MDA concentrations increased significantly at the nadir day of the first cycle, and remained thereafter at this higher level, similar to the MDA levels before the start of supplementation (Table 3). In the placebo group, MDA levels did not change significantly throughout the whole chemotherapy treatment period.

Twenty two (46%) of the 48 patients did not drink the study beverage throughout the whole study period. The reasons for stopping were a combination of dislike of the taste of the milky, vanilla-flavoured beverage over a long period of time, nausea and upper abdominal

Table 2
Plasma concentrations of antioxidant micronutrients and MDA before and 7 days after the start of supplementation[a]

Parameters	Normal values	Placebo ($n = 23$)			Supplementation ($n = 25$)			
		Day −7[b]	Day 1[c]	P[d] value	Day −7[b]	Day 1[c]	P[d] value	P[e] value
Total vitamin C	11–100 μmol/l	58.6 ± 48.7	51.5 ± 35.6	NS	48.1 ± 34.7	121.3 ± 52.7	<0.001	<0.001
Oxidised vitamin C	(μmol/l)	7.4 ± 4.6	6.5 ± 3.7	NS	5.4 ± 3.2	14.7 ± 9.3	<0.001	<0.001
Reduced vitamin C	(μmol/l)	51.2 ± 47.9	45.0 ± 33.5	NS	42.7 ± 32.3	106.6 ± 52.2	<0.001	<0.001
Reduced/oxidised vitamin C		9.2 ± 15.1	7.1 ± 4.1	NS	7.6 ± 4.2	9.0 ± 4.4	NS	NS
Vitamin E	18.0–43.1 μmol/l	26.6 ± 8.7	26.4 ± 9.2	NS	26.0 ± 7.6	60.0 ± 25.1	<0.001	<0.001
Cholesterol	3.9–7.3 mmol/l	4.6 ± 1.1	4.5 ± 0.9	NS	5.2 ± 1.9	5.1 ± 1.7	0.374	0.106
Triglycerides	0.80–1.94 mmol/l	1.1 ± 0.5	1.1 ± 0.5	NS	1.1 ± 0.6	1.3 ± 0.8	0.046	NS
Vitamin E/lipids	(μmol/mmol)	4.8 ± 1.6	4.8 ± 1.5	NS	4.3 ± 1.0	9.5 ± 2.7	<0.001	<0.001
Selenium	0.8–1.4 μmol/l	1.1 ± 0.3	1.0 ± 0.3	NS	1.3 ± 0.4	1.5 ± 0.5	<0.001	<0.001
Malondialdehyde (MDA)	0.5–1.3 μmol/l	1.1 ± 0.4	1.2 ± 0.5	NS	1.4 ± 0.7	1.2 ± 0.4	NS	NS

[a] Data represent means ± SD.
[b] Before supplementation.
[c] After 7 days of supplementation, just before the start of chemotherapy.
[d] P-value for comparison between values of day −7 and day 1 within the same randomisation group (paired-samples Student's t-test). NS, non-significant.
[e] P-value for comparison between values of day 1 of both randomisation groups (independent-samples Student's t-test). NS, non-significant.

Table 3
Plasma concentrations of antioxidant micronutrients and MDA during the chemotherapy period

Parameters	Normal values	Placebo ($n=23$)					Supplementation ($n=25$)					
		Cycle 1			Cycle 2	Cycle 4	Cycle 1				Cycle 2	Cycle 4
		First day	Nadir day	P^a value	First day	First day	First day	Nadir day	P^a value	P^b value	First day	First day
Total vitamin C	11–100 μmol/l	51.5 ± 35.6	29.1 ± 13.9	< 0.001	33.6 ± 22.5	40.3 ± 27.5	121.3 ± 52.7	61.6 ± 44.6	<0.001	<0.001	96.7 ± 60.0	78.7 ± 45.9
Oxidised vitamin C	(μmol/l)	6.5 ± 3.7	5.6 ± 2.7	NS	5.2 ± 3.5	5.8 ± 5.2	14.7 ± 9.3	7.7 ± 3.3	<0.001	0.027	8.7 ± 4.4	11.5 ± 9.8
Reduced vitamin C	(μmol/l)	45.0 ± 33.5	24.5 ± 12.7	<0.001	28.4 ± 3.5	34.5 ± 24.0	106.6 ± 52.2	55.8 ± 43.4	<0.001	<0.001	88.0 ± 58.5	67.6 ± 41.2
Reduced/oxidised vitamin C		7.1 ± 4.1	4.6 ± 2.1	0.01	5.7 ± 2.6	6.4 ± 3.2	9.0 ± 4.4	7.9 ± 5.3	NS	0.012	12.0 ± 11.6	7.3 ± 3.9
Vitamin E	18.0–43.1 μmol/l	26.4 ± 9.2	20.4 ± 7.7	<0.001	30.2 ± 10.2	34.7 ± 9.2	60.0 ± 25.1	33.9 ± 9.6	<0.001	<0.001	55.0 ± 31.4	52.0 ± 24.4
Cholesterol	3.9–7.3 mmol/l	4.5 ± 0.9	4.5 ± 0.8	NS	4.9 ± 1.0	5.6 ± 1.1	5.1 ± 1.7	4.7 ± 1.3	NS	NS	5.3 ± 1.3	5.8 ± 1.5
Triglycerides	0.80–1.94 mmol/l	1.1 ± 0.5	1.2 ± 0.6	NS	1.5 ± 0.5	1.9 ± 0.7	1.3 ± 0.8	1.0 ± 0.5	NS	NS	1.8 ± 1.5	1.8 ± 0.8
Vitamin E/lipids	(μmol/mmol)	4.8 ± 1.5	3.8 ± 1.3	<0.001	4.7 ± 1.2	4.6 ± 0.7	9.5 ± 2.7	6.1 ± 1.9	<0.001	<0.001	7.8 ± 3.6	7.0 ± 3.3
Selenium	0.8–1.4 μmol/l	1.0 ± 0.3			0.9 ± 0.3	0.9 ± 0.2	1.5 ± 0.5				1.4 ± 0.4	1.3 ± 0.4
Malondialdehyde	0.5–1.3 μmol/l	1.2 ± 0.5	1.3 ± 0.5	NS	1.3 ± 0.6	1.2 ± 0.5	1.2 ± 0.4	1.4 ± 0.5	0.026	NS	1.4 ± 0.8	1.2 ± 0.6

[a] P-value for comparison between values of the first day and the ''nadir day'' of the first chemotherapy cycle (paired Student's t-test). NS; non-significant.
[b] P-value for comparison of values of the ''nadir days'' of the first chemotherapy cycle between both study groups (independent-samples Student's t-test). NS; non-significant.

discomfort, particularly during the days of cytostatic drug administration. Eleven, three, and eight of the 48 patients stopped drinking the beverage after the first, second, and third chemotherapy cycles, respectively. In the placebo group, 6 (26%) patients and in the intervention group 16 (64%) were non-compliant (χ^2 test; $P = 0.009$). No other than gastro-intestinal side-effects related to supplementation of vitamin C, E, or selenium, e.g. vitamin C-related renal oxalate stones, vitamin E-related coagulopathy or selenium-associated skin or nail abnormalities, were observed in patients in the intervention arm.

The primary and secondary outcome measures of the study are shown in Table 4. After three cycles of chemotherapy and two months after cessation of chemotherapy, a significant decrease in renal and hearing function compared with baseline values was observed in both patient groups. No significant differences were found between the placebo and supplementation group with respect to the decrease in the creatinine clearance and loss of high-tone hearing at the various time points measured during and following chemotherapy. In both patient groups, haematological cell counts markedly decreased at nadir days following each chemotherapy cycle (data not shown), but the (partial) recovery from chemotherapy induced anaemia, leucocytopenia and thrombocytopenia at the start of each subsequent cycle and two months after cessation of chemotherapy did not differ significantly between the study groups (Table 4). Furthermore, in both groups plasma magnesium concentrations decreased significantly during chemotherapy and returned to baseline values following chemotherapy. The nadir plasma magnesium concentrations did not differ significantly between the study groups (data not shown).

A correlation analysis was performed to investigate the relationship between antioxidant micronutrients, MDA plasma concentrations and the primary outcome measures of the study. Although the plasma concentrations of the individual supplemented micronutrients did not correlate significantly with nephrotoxity or ototoxicity (data not shown), the reduced/oxidised vitamin C ratio as measured on the first day of the first and fourth chemotherapy cycles correlated with the loss of high-tone hearing after one and three chemotherapy cycles, respectively ($r = 0.326, P = 0.04$ and $r = 0.464, P = 0.019$, respectively; Fig. 2(a) and (b)). In addition, patients' maximal level of MDA in the first chemotherapy cycle correlated with loss of renal function after three cycles of chemotherapy ($r = -0.503, P = 0.009$; Fig. 3).

If patients were divided into two groups based on their levels of reduced/oxidised vitamin C ratios (a high ratio indicating decreased and a low ratio indicating increased oxidative stress), MDA concentrations or overall antioxidant micronutrient scores, a significant

Table 4
Primary and secondary outcome measures determined at the start of each chemotherapy cycle and 2 months after cessation of chemotherapy[a,b]

Parameters	Placebo ($n = 23$)				Supplementation ($n = 25$)			
	Cycle 1	Cycle 2	Cycle 4	2 months	Cycle 1	Cycle 2	Cycle 4	2 months
Measured creatinine clearance (ml/s)	1.73 ± 0.72	1.72 ± 0.58	1.65 ± 0.63	1.43 ± 0.47[c]	1.97 ± 0.58	2.02 ± 0.78	1.77 ± 0.63	1.65 ± 0.65[c]
Conduction threshold at 8.0 dB	19.5 ± 16.3	25.1 ± 21.5[c]	31.0 ± 23.3[c]	32.2 ± 25.7[c]	27.1 ± 19.0	28.8 ± 19.2	37.5 ± 22.5[c]	42.5 ± 25.8
Blood haemoglobin (mmol/l)	8.1 ± 1.2	7.2 ± 0.8[c]	6.8 ± 1.0[c]	7.8 ± 1.1[c]	8.4 ± 0.9	7.4 ± 0.8[c]	6.8 ± 0.7[c]	7.6 ± 1.1[c]
White blood cell count (10^9cells/l)	8.1 ± 3.6	8.6 ± 17.7	4.9 ± 2.8[c]	5.9 ± 1.8[c]	7.3 ± 1.9	4.4 ± 1.8[c]	6.2 ± 5.6	5.7 ± 2.1[c]
Platelets (10^9cells/l)	322 ± 114	386 ± 167	294 ± 115	231 ± 66[c]	277 ± 95	332 ± 187	267 ± 108	228 ± 110[c]

[a] Data represent means ± SD.
[b] No significant differences were found between the study arms for all parameters.
[c] $P < 0.05$ for comparison within each study arm between values of the first chemotherapy cycle and the subsequent time points (paired Student's t-test).

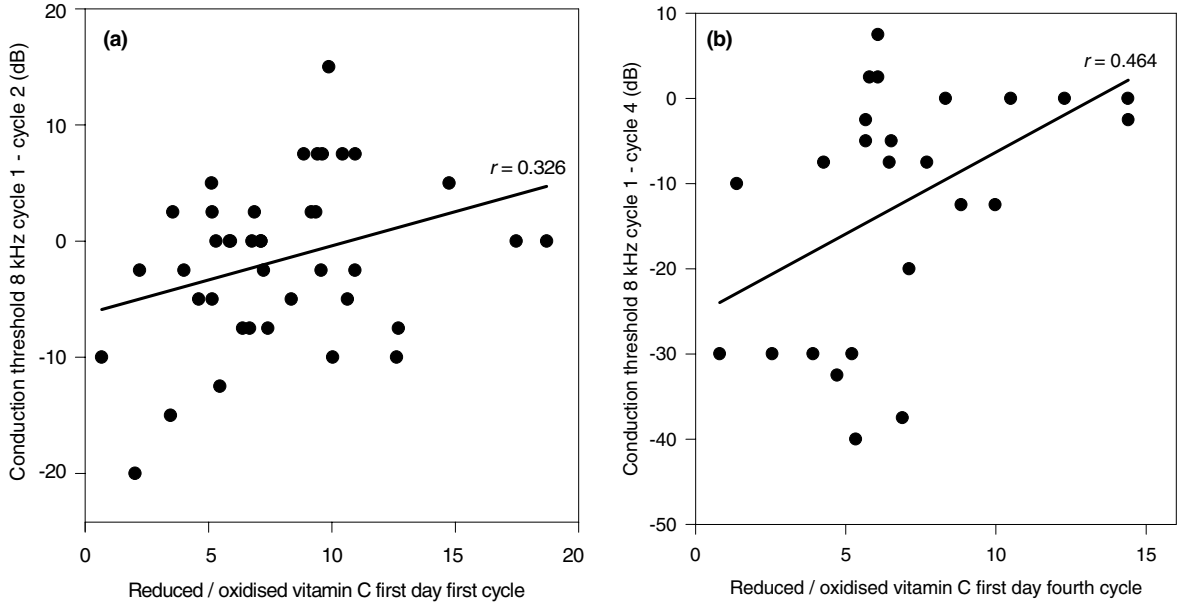

Fig. 2. (a) Correlation scatter plot of the reduced/oxidised vitamin C ratio on day 1 of the first cycle and changes in the conduction threshold at 8 kHz between the first and the second chemotherapy cycles. Scatters represent values of individual patients. Values <0 indicate increased conduction thresholds, i.e. loss of high tone hearing after the first chemotherapy cycle; values >0 represent decreased conduction thresholds, i.e. improved high tone hearing. The line represents the first order regression line (r). The correlation between the reduced/oxidised vitamin C ratio on day 1 of the first cycle and changes in conduction threshold at 8 kHz is expressed by the correlation coefficient $r = 0.326$ ($P = 0.04$). (b) Correlation scatter plot of the reduced/oxidised vitamin C ratio on day 1 of the fourth cycle and changes in the conduction threshold at 8 kHz between the first and the fourth chemotherapy cycles. Scatters represent values of individual patients. Values are as in Fig. 2(a). The correlation between the reduced/oxidised vitamin C ratio on day 1 of the first cycle and changes in conduction threshold at 8 kHz is expressed by the correlation coefficient $r = 0.464$ ($P = 0.019$).

correlation was found between the loss in renal and hearing function as measured two months after completion of chemotherapy and these three parameters. Patients with a reduced/oxidised vitamin C ratio of $\geqslant 7.5$ at the start of chemotherapy had significantly less loss of high-tone hearing and less decrease in creatinine clearance after chemotherapy than patients with a ratio of <7.5 (decrease in conduction threshold at 8.0 kHz at two months post-chemotherapy 4.2 ± 10.1 vs. 21.6 ± 20.1 dB; $P = 0.016$; decrease in creatinine clearance $2.1 \pm 15.1\%$ vs. $17.0 \pm 20.2\%$; $P = 0.017$). Similarly, patients with MDA concentrations <1.5 μmol/l at day one of the first chemotherapy cycle had significantly less loss in renal function than patients with a MDA concentration of $\geqslant 1.5$ μmol/l (decrease in creatinine clearance $3.8 \pm 15.2\%$ vs. $17.4 \pm 16.8\%$; $P = 0.046$). Patients with the highest micronutrient antioxidant score (score = 6) at the start of chemotherapy also had significantly less loss of high-tone hearing than patients with a low overall score (score $\leqslant 5$; decrease in conduction threshold at 8.0 kHz 2.8 ± 5.3 vs. 14.4 ± 13.7 dB; $p = 0.028$).

4. Discussion

In this study, we have investigated the role of supplementation of antioxidant micronutrients in the prevention of cisplatin-related toxicity. Despite significantly higher mean plasma concentrations of the supplemented micronutrients during the entire chemotherapy period, patients in the intervention group did not have less nephrotoxicity, ototoxicity or bone marrow toxicity than control patients. However, independent of whether or not patients received supplementation or placebo, patients who seemed to have a better oxidative defense status based on their higher plasma levels of vitamin C, E and selenium (“antioxidant micronutrient score”), higher reduced/oxidised vitamin C ratio and/or lower MDA levels at the start and during the first cycle of chemotherapy, had less loss of renal and hearing function. No supplementation-related toxicity was observed and clinical remissions were equally divided in both groups of patients, indicating that supplementation with antioxidant did not have an adverse effect on the patient’s clinical response. Patients’ compliance to the beverages and, in particular, the supplementation beverage was poor with 46% of all patients and 64% of the intervention patients failing to drink the study beverage throughout the whole study period.

The failure of intervention in the current study to protect against chemotherapy-induced toxicity may be explained in various ways. The lack of compliance in this study may be a major determinant explaining the failure to protect against oxidative damage by chemotherapy. The observed high rate of poor compliance is in

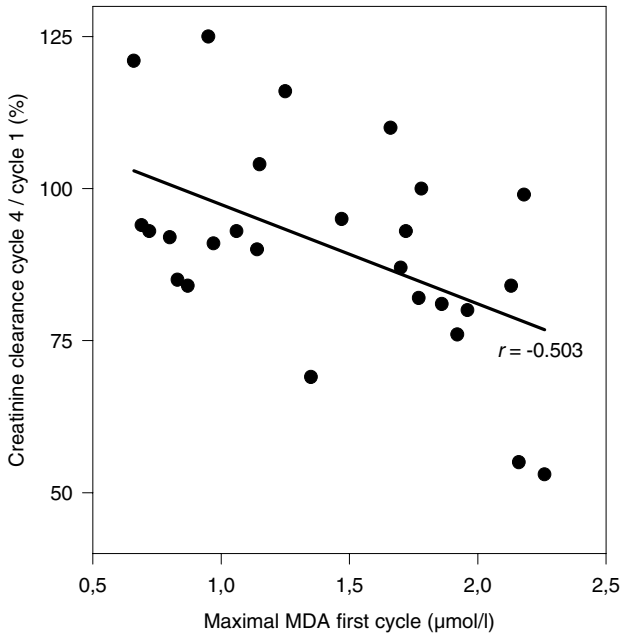

Fig. 3. Correlation scatter plot of maximal MDA during first cycle and changes in creatinine clearance between the first and the fourth chemotherapy cycles. Scatters represent values of individual patients. The vertical axis represents the mean creatinine clearance on day 1 of the fourth chemotherapy cycle as percentage of the mean creatinine clearance on the first day of the first chemotherapy cycle; values <100% indicate a decrease of the creatinine clearance after three chemotherapy cycles (loss in renal function) compared with pretreatment creatinine clearance. The line represents the first order regression line (r). The correlation between maximal MDA and changes in creatinine clearance is expressed by the correlation coefficient $r = -0.503$ ($P = 0.009$).

accordance with other reports concerning non-compliance in the treatment of malignancies and other diseases [16–19]. The higher rate of non-adherence to the trial beverage in the intervention group compared with the placebo group may be attributed to the acidity of vitamin C, that can cause gastrointestinal upset such as nausea, abdominal cramps and diarrhoea when given at a daily dosage of 1 g or more [20]. This is in accordance with the higher frequency of reported abdominal discomfort by patients in the intervention arm. In addition, the high plasma antioxidant micronutrients concentrations obtained following one week of supplementation were markedly decreased immediately after the first chemotherapy infusion and did not return to prechemotherapy levels during subsequent chemotherapy cycles. The rapid decline in plasma antioxidants may result from consumption of antioxidants caused by chemotherapy-induced oxidative stress and, in part, to renal loss of vitamin C, a water-soluble and small molecular weight antioxidant [7]. The observed fluctuations of plasma MDA concentrations at the time points measured during chemotherapy in the supplementation, but not in the placebo group, are not well understood.

A higher daily dose or longer period of supplementation prior to the start of chemotherapy and the addition of other antioxidants involved in the protection against oxidative damage might have resulted in higher antioxidant micronutrient concentrations in both plasma and body tissues and synergistic interactions between the antioxidants, leading to effective prevention against free radical-mediated organ damage. The significant correlations between the reduced/oxidised vitamin C ratio and the lipid peroxidation product MDA, two biomarkers reflecting the net effect of the antioxidative defense system, and the cisplatin-induced toxicity underscore that supplementation with (higher dosages) of multiple antioxidants -if administered in an optimal way- could, in principal, ameliorate the free radical-mediated side-effects of specific cytostatic drugs.

Many other agents have been studied preclinically in an attempt to reduce the toxicities associated with cisplatin and other cytostatic drugs that are, in part, mediated by free radical formation [3,21–23]. Most of the clinical experience obtained thus far has been using synthetic antioxidant agents and a few of these compounds have been extensively evaluated in cancer patients. Of the investigated drugs, thiol-containing agents that are able to scavenge free radicals and bind to aquated platinum molecules, are one of the most promising classes of protectants against cisplatin-induced toxicities and have been studied in various phase II and III trials. Despite encouraging initial clinical studies, glutathione and diethyldithiocarbamate (DDTC) have not fulfilled expectations as protectors against cisplatin toxicities and patients receiving DDTC in fact suffered from significantly more nephrotoxicity than patients in the placebo arm [6,24,25]. Amifostine is currently the only thiol-containing agent that has been shown to reduce nephrotoxicity and peripheral neuropathy in randomised trials [26,27], although in some trials only a reduction in the incidence of hypomagnesaemia and subclinical peripheral neuropathy was observed [27]. Amifostine-related side-effects such as severe hypotension, nausea, flushing and sneezing were frequently observed, precluding the frequent use of this protectant. Although protection against the side-effects of cisplatin by natural antioxidants such as vitamin C and E has been demonstrated in various animal studies [3,28], these natural antioxidants have been, until now, rarely investigated in the clinic. Pace and colleagues [29] recently performed a clinical study in which they evaluated the neuroprotective effect of vitamin E in 27 patients treated with cisplatin chemotherapy. In the 13 evaluable patients who were randomised to receive 300 mg/day vitamin E orally during cisplatin chemotherapy, the incidence and severity of neurotoxicity were significantly lower than in the 14 patients who were treated with cisplatin chemotherapy alone.

In summary, although no significant differences in chemotherapy-induced organ toxicity were observed between the study arms, as analysed according to an intention-to-treat principle, supplementation of the combination of vitamin C, vitamin E and selenium led to markedly increased plasma antioxidants levels. However, the enhanced plasma antioxidant concentrations were not maintained during chemotherapy, most likely as a result of antioxidant consumption or renal loss of antioxidants and a lack of compliance to the study beverages. Our observation that parameters reflecting the antioxidative defense status of the patient significantly correlated with cisplatin-induced toxicity, underscore the potential of adequate supplementation with antioxidants in providing protection against organ toxicity. This observation has led us to initiate a clinical study in which patients undergoing cisplatin- or doxorubicin-based chemotherapy are now randomised to receive an oral combination of vitamin C, E and selenium and eight other antioxidants.

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