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Serum Alpha-tocopherol, Retinol and Neopterin during Paclitaxel/Carboplatin Chemotherapy

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Abstract. *Background: Disorders of antioxidant balance are considered to be involved in the toxicity associated with radiotherapy or chemotherapy. Patients and Methods: Serum alpha-tocopherol and retinol were determined, by high performance liquid chromatography, before and during therapy with a combination of paclitaxel and carboplatin in 28 patients with breast and ovarian cancer. Serum neopterin and cholesterol were measured using a radioimmunoassay and enzymatic colorimetric method, respectively. Results: Compared to pretreatment concentrations, a significant increase was observed in serum alpha-tocopherol and retinol concentrations during therapy that was associated with decreased serum neopterin concentrations. Serum alpha-tocopherol concentrations were significantly higher during therapy in patients who did not experience serious toxicity. Conclusion: An increase in alpha-tocopherol and retinol during therapy with combination paclitaxel/carboplatin may be explained by inhibition of systemic immune activation secondary to control of the tumor with effective chemotherapy. Lower alpha-tocopherol concentrations were associated with the toxicity of therapy.*

Administration of cytotoxic drugs is associated with oxidative stress (1, 2), and disorders of antioxidant balance may be involved in the toxicity associated with anticancer treatment. Vitamin E represents a major antioxidant in the serum (3). Retinol is a major circulating form of vitamin A that also has antioxidant activity (3).

Another molecule associated with antioxidant balance is neopterin, an unconjugated pteridine synthesized from guanosine triphosphate (4). Human monocytes/macrophages produce significant quantities of neopterin when stimulated with interferon-γ, and neopterin is regarded as an indicator of systemic immune activation. Increased urinary and serum neopterin concentrations have been reported in patients with different primary tumors, including epithelial ovarian carcinoma (EOC) or metastatic breast carcinoma (4-6). An association has been detected between the course of the disease and neopterin levels. Neopterin concentrations decreased after successful therapy, but increased during disease progression. Elevated neopterin concentrations were limited to patients with active disease, and normal neopterin levels were reported in patients without evidence of disease activity (4). Increased urinary neopterin has also been associated with poor prognosis both in EOC (4, 5) and breast carcinoma (7).

The term vitamin E denotes several naturally occurring tocopherols and tocotrienols, but alpha-tocopherol is responsible for most vitamin E activity in animal tissues (3). Disorders of antioxidant balance involving vitamin E are thought to be involved in the toxicity associated with radiotherapy (8, 9), or chemotherapy (10), and a decrease in serum alpha-tocopherol has been observed during systemic chemotherapy (1, 2, 11, 12). Retinol plays an essential role in many physiological functions, including vision, growth, development, differentiation and the immune response (3).

The combination of platinum derivatives (cisplatin or carboplatin) with paclitaxel represents currently the standard front line regimen for patients with advanced EOC after demonstration of superior survival in randomized clinical trials (13, 14). This combination is also active in patients with metastatic breast carcinoma (15). The combination of paclitaxel and carboplatin has a relatively favorable toxicity profile. Although this combination is one of the most commonly used in medical oncology, little is known about its effect on antioxidant balance.

In the present study, the serum alpha-tocopherol, retinol, neopterin and cholesterol concentrations in breast and

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ovarian cancer patients treated with paclitaxel and platinum chemotherapy have been investigated. The principal aim was to investigate changes of serum retinol, alpha-tocopherol and neopterin during chemotherapy with combination paclitaxel/carboplatin. As the concentrations of alpha-tocopherol are dependent on serum lipid levels, serum cholesterol was determined to estimate the alpha-tocopherol/cholesterol ratio. A secondary aim of the study was to compare these parameters with the presence or absence of serious (grade 3 or higher) toxicity.

Patients and Methods

Twenty-eight women, mean age 56±10 (range 34-74) years, treated with paclitaxel and carboplatin, were included in the present study. Nine patients had ovarian cancer (primary EOC 7 patients and Krukenberg tumors 2 patients), and 19 patients had breast carcinoma. Three of the patients with primary EOC also had a history of breast cancer. Four patients were chemotherapy-naïve and 24 patients had a history of previous chemotherapy. Sixteen patients were treated with a combination of paclitaxel (175 mg/m^2) and carboplatin (area under the curve 6) administered every 3 weeks. Twelve patients were treated with a weekly regimen of paclitaxel (90 mg/m^2) with carboplatin (area under the curve 2) with (8 patients) or without (4 patients) trastuzumab (4 mg/kg loading dose, then 2 mg/kg weekly). The study protocol was approved by the institutional ethical committee and the patients signed informed consent. No supplementary vitamins were prescribed, and the patients were advised to take a normal diet. The toxicity was assessed using Common Terminology Criteria for Adverse Events version 3.0 (16). Serious toxicity was defined as of grade 3 or higher.

Serum alpha-tocopherol and retinol were determined before and during the therapy by high performance liquid chromatography as described previously (17). Blood samples were drawn from a peripheral vein after an overnight fast. The samples were transferred immediately to the laboratory, centrifuged (1600 xg, 10 minutes, 16˚C), and the serum was frozen at –20˚C until analysis. In the liquid-liquid extraction procedure, 500 μl of serum was deproteinized by cool ethanol denatured with 5% methanol (500 μl, 5 minutes, 4˚C). Subsequently, 2500 μl of n-hexane was added to this mixture and extracted for 5 minutes by a vortex apparatus. After centrifugation (1600 xg, 10 minutes, 0˚C), the aliquot (2000 μl) of the clean extract was separated and evaporated under nitrogen (60˚C). The residue was dissolved in 400 μl methanol and analysed by reversed-phase high performance liquid chromatography using external standard calibration. The analyses were performed using the Perkin Elmer high performance liquid chromatography set (Norwalk, CT, USA) comprising a LC 200 pump, a LC 200 autosampler, LC Column Oven 101 thermostat and LC 235C Diode Array Detector attached to the Perkin Elmer Turbochrom Chromatography Workstation version 4.1. The separation of alpha-tocopherol and retinol was performed using a Chromolith Performance RP-18e, 100x4.6 mm monolithic columns (Merck, Darmstadt, Germany). As the mobile phase 100% methanol was used at a flow rate of 2.5 ml/min^{-1} and column pressure of 3.3 MPa. A block heater LC Oven 101 (Perkin Elmer) was utilized to keep the analytical column temperature at 25˚C. The injection volume was 50 μl. The detection of alpha-tocopherol and retinol was carried out at 295 nm and at 325 nm, respectively.

Serum neopterin was determined with radioimmunoassay using a commercial kit (Brahms, Hennigsdorf, Germany) according to the instructions of the manufacturer. Serum cholesterol was determined by an enzymatic colorimetric test (with cholesterol esterase and cholesterol oxidase) using a modular analyzer with a commercial kit according to the manufacturer's instructions (Roche, Mannheim, Germany).

The significance of differences during the therapy compared to pretreatment values was studied by Wilcoxon paired test, the differences of concentrations in patients with or without toxicity were evaluated by Mann-Whitney *U*-test, and correlations were analyzed with Spearman's rank correlation coefficient using NCSS 2001 software (Number Cruncher Statistical Systems, Kaysville, UT, USA). The decision on statistical significance was based on $p<0.05$ level.

Results

The serum samples were obtained before the start of therapy (baseline, visit 1), during the first cycle of chemotherapy (one week after the start of treatment, visit 2), at the end of the first or second cycle of chemotherapy (visit 3), and before subsequent chemotherapy cycles (visit 4). The median number of measurements during therapy was 3 (range 1-8). Compared to pretreatment concentrations (Table I), a significant increase was observed in alpha-tocopherol and retinol concentrations, and alpha-tocopherol/cholesterol ratios throughout the course of therapy. A slight increase in serum cholesterol was observed that did not reach statistical significance. In contrast, a trend of decreased serum neopterin was observed that reached significance at visit 3. Similar results were observed when means of all measurements subsequent to visit 2 were evaluated.

Ten patients experienced serious (grade 3 or higher) toxicity during the first 6 weeks of therapy (leukopenia or neutropenia 5 patients, diarrhea 2 patients, nausea 2 patients, anemia 1 patient). Serum alpha-tocopherol concentrations were significantly higher in patients who did not experience serious toxicity at visits 2 and 3 and serum retinol was significantly higher at visit 2 (Table II).

Baseline serum concentrations of cholesterol significantly correlated with retinol ($r_s=0.54$; $p=0.005$) and alpha-tocopherol ($r_s=0.43$; $p=0.02$). A correlation was observed between retinol and alpha-tocopherol ($r_s=0.43$; $p=0.02$), and baseline serum neopterin correlated inversely with cholesterol ($r_s=–0.47$; $p=0.02$).

Discussion

The significant increase of serum alpha-tocopherol and retinol concentrations during paclitaxel/carboplatin combination chemotherapy observed in the present study may seem rather unexpected in the light of earlier reports of decreased alpha-tocopherol concentrations during systemic chemotherapy that were associated with oxidative stress induced by the therapy (1, 2, 11, 12). Moreover,

Table I. *Serum retinol, alpha-tocopherol and neopterin levels before, during and after chemotherapy treatment*

	Visit 1 (baseline)	Visit 2 (during the first cycle)	Visit 3 (at the end of first or second cycle)	Visit 4 (subsequent to visit 3)	Mean of all measurements subsequent to visit 2
Time from the start of therapy (days)	0	8±4	23±10	46±34	-
Retinol (μmol/l)	1.27±0.53 (0.12-2.24)	1.56±0.62*** (0.31-2.65)	1.67±0.66*** (0.64-3.44)	1.76±0.64** (0.65-3.63)	1.64±0.62*** (0.64-3.33)
Alpha-tocopherol (μmol/l)	23.6±5.2 (12.5-35.5)	26.4±6.4** (17.5-40.9)	27.5±6.1*** (17.7-40.9)	27.7±6.4** (18.3-41.5)	27.4±5.6** (19.7-40.2)
Neopterin (nmol/l)	8.4±4.3 (3.8-17.9)	8.6±4.4 (3.4-17.2)	7.2±4.1* (2.7-19.6)	7.0±4.4 (3.3-23.5)	7.0±3.6 (3.0-19.6)
Cholesterol (mmol/l)	5.29±0.96 (3.96-7.67)	5.35±1.05 (3.79-7.92)	5.70±1.03 (3.39-7.62)	5.79±1.06 (4.12-7.70)	5.74±0.94* (3.96-7.69)
Alpha-tocopherol/cholesterol (mmol/mol)	4.49±1.00 (3.05-7.17)	5.05±0.81** (3.69-6.61)	4.82±0.74** (3.05-6.61)	4.87±0.76* (3.57-6.11)	4.81±0.66** (3.46-6.29)

Shown are means±standard deviations (range). Wilcoxon paired test, *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to baseline.

Table II. *Serum analysis before therapy, during and after the first cycle of chemotherapy in patients with or without serious toxicity.*

Parameter	No serious toxicity (n=18)	Serious toxicity (n=10)	*p*-value
Retinol-visit 1(μmol/l)	1.38±0.47 (0.59-2.24)	1.07±0.59 (0.12-2.08)	0.12
Retinol-visit 2 (μmol/l)	1.73±0.59 (0.63-2.65)	1.26±0.59 (0.31-2.20)	0.047
Retinol-visit 3 (μmol/l)	1.82±0.69 (1.11-3.44)	1.39±0.51 (0.64-2.02)	0.20
Alpha-tocopherol-visit 1 (μmol/l)	25.0±4.5 (17.4-35.5)	20.9±5.6 (12.5-29.0)	0.08
Alpha-tocopherol -visit 2 (μmol/l)	28.4±6.7 (17.5-40.9)	22.9±4.3 (18.3-30.2)	0.042
Alpha-tocopherol-visit 3 (μmol/l)	29.5±6.4 (17.7-40.9)	23.9±3.7 (18.2-29.5)	0.020
Neopterin-visit 1(nmol/l)	7.6±3.3 (3.8-15.0)	9.9±5.7 (4.2-17.9)	0.48
Neopterin-visit 2 (nmol/l)	7.2±3.3 (3.4-13.6)	10.8±5.1 (5.0-17.2)	0.10
Neopterin-visit 3 (nmol/l)	6.3±3.4 (2.7-16.6)	8.9±4.9 (3.8-19.6)	0.07
Cholesterol-visit 1 (mmol/l)	5.52±1.05 (3.96-7.67)	4.81±0.54 (4.11-5.61)	0.11
Cholesterol-visit 2 (mmol/l)	5.63±1.21 (3.79-7.92)	4.94±0.60 (4.00-6.07)	0.19
Cholesterol-visit 3 (mmol/l)	5.90±1.07 (3.39-7.62)	5.35±0.90 (4.11-6.93)	0.23
Alpha-tocopherol/cholesterol-visit 1 (mmol/mol)	4.66±0.93 (3.63-7.17)	4.15±1.12 (3.05-6.51)	0.13
Alpha-tocopherol/cholesterol-visit 2 (mmol/mol)	5.33±0.81 (3.95-6.61)	4.64±0.62 (3.69-5.39)	0.09
Alpha-tocopherol/cholesterol-visit 3 (mmol/mol)	5.00±0.74 (3.96-6.61)	4.52±0.68 (3.05-5.15)	0.23

Shown are means±standard deviations (range) and respective *p*-values (Mann-Whitney *U*-test). Visit 1, before therapy; visit 2, during the first cycle of chemotherapy; visit 3, at the end of the first or second cycle of chemotherapy.

administration of paclitaxel/carboplatin chemotherapy is associated with small bowel dysfunction, and disturbance of the small bowel is accompanied by low serum concentration of retinol (18, 19). Despite this, a moderate, but statistically significant increase in serum retinol was observed.

Only a minor increase of serum cholesterol was observed, while an increase of serum alpha-tocopherol was accompanied by an increased alpha-tocopherol/cholesterol ratio. Thus, the change of serum alpha-tocopherol was not due to changes in serum lipid levels. It has been demonstrated previously that serum concentrations of alpha-tocopherol and retinol were significantly decreased in patients with advanced cancer (20, 21). It has also been shown that the decrease of serum alpha-tocopherol and retinol concentrations correlated with the systemic inflammatory response (20, 22). The synthesis of retinol binding protein was decreased by pro-inflammatory cytokines (23). Moreover, anti-inflammatory therapy (ibuprofen) in patients with advanced cancer led to an increase in serum carotenoids (21). The increase in serum alpha-tocopherol and retinol observed after administration of paclitaxel/carboplatin chemotherapy in this study was therefore probably linked to suppression of the systemic inflammatory response by the chemotherapy.

Although both paclitaxel and cisplatin have been demonstrated to activate macrophages *in vitro* (24, 25), and we have recently reported an increase of urinary neopterin in breast cancer patients treated with a combination of paclitaxel and doxorubicin (6), in the present study a decrease rather than an increase of serum neopterin was observed after administration of paclitaxel/ carboplatin chemotherapy. This was in agreement with earlier data showing that the reduction of tumor burden may result in lower neopterin concentrations (26). Baseline serum neopterin was above the normal range in most patients, and the decrease in serum neopterin may have reflected suppression of the systemic inflammatory response associated with effective anti-tumor therapy. An increase in serum alpha-tocopherol has also been reported recently after hyperthermia (27). Serum retinol is bound to retinol binding protein (3), and serum levels of retinol binding protein are markedly increased in patients with renal dysfunction (28). Thus, the mild elevation of retinol observed in the present study could also have resulted from an increase of retinol binding protein secondary to subclinical renal toxicity induced by the platinum derivatives.

While all the patients in the current study had assessment of alpha-tocopherol and retinol at baseline and during the first chemotherapy cycle, the number of subsequent measurements was different because of the differences in the duration of therapy in individual patients. Therefore, in addition to the evaluation of the results obtained at the end of the first or second cycle, an analysis was also performed of the means of sequential measurements as described by Matthews *et al.* (29), and alpha-tocopherol and retinol concentrations assessed at the end of the first or second cycle as well as the means of sequential measurement showed similar results.

In earlier studies, low vitamin E intake was associated with toxicity of chemotherapy in children with acute lymphoblastic leukemia (30). The administration of vitamin E has been shown to alleviate some side effects of radiotherapy (8) or chemotherapy (10). Although serial monitoring of vitamin E levels may be a necessary prerequisite for any therapeutic use of this antioxidant vitamin, serum vitamin E is not being routinely measured in cancer patients. In most reports published to date, alpha-tocopherol has been investigated in epidemiological studies in relation to cancer risk. For example, lower vitamin E concentrations have been reported in patients with breast cancer (31). Lower alpha-tocopherol concentrations during the first 6 weeks of therapy in the present study were associated with significant toxicity. Low serum alpha-tocopherol could be a consequence of chemotherapy toxicity resulting in lower food intake. Lower serum alpha-tocopherol could also increase the risk of toxicity or reflect the presence of other factor(s) associated with a higher risk of toxicity. In fact a trend of lower baseline alpha-tocopherol was observed in patients who subsequently experienced toxicity. However, these results should be regarded as preliminary, and only an interventional study of vitamin E supplementation in a larger cohort could clarify whether decreased serum alpha-tocopherol is a consequence or cause of chemotherapy toxicity.

We conclude that, contrary to expectations based on previous reports, serum alpha-tocopherol and retinol increased significantly during the therapy with combination paclitaxel/carboplatin, but lower alpha-tocopherol concentrations were observed during the therapy in patients who experienced serious toxicity. An increase in serum alpha-tocopherol and retinol after paclitaxel/carboplatin chemotherapy seems to be associated with the suppression of the systemic immune and inflammatory response secondary to tumor control induced by chemotherapy.

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