Serum Retinol, Alpha-Tocopherol and Systemic Inflammatory Response in Metastatic Colorectal Carcinoma Patients Treated with Combination Chemotherapy and Cetuximab

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Summary  Cetuximab is a chimeric antibody registered for the therapy of advanced colorectal carcinoma. Cancer and anticancer therapy are associated with oxidative stress, and disorders of antioxidant balance may be involved in the toxicity associated with anticancer treatment. The aim of the present study was to investigate the changes of serum retinol, alpha-tocopherol and C-reactive protein during the first month of treatment with cetuximab and chemotherapy. Twenty-five consecutive patients with metastatic colorectal carcinoma treated with a combination of chemotherapy and cetuximab were included in the present study. Serum retinol and alpha-tocopherol were determined by high-performance liquid chromatography and serum C-reactive protein was determined using commercial kits. Significant correlation was observed between baseline concentrations of retinol and C-reactive protein ($r_s = -0.54$, $p<0.01$). Median survival of patients who had baseline serum retinol below 1.25 $\mu$mol/L was 10 mo compared to 18 mo for patients who had serum retinol equal or above 1.25 $\mu$mol/L ($p<0.05$); median survival of patients who had serum C-reactive protein below 24 mg/L was significantly longer compared to patients with C-reactive protein levels equal or above 24 mg/L (18 vs. 7 mo, $p<0.05$), but no difference in survival was observed based on alpha-tocopherol levels. Twenty-two patients had evaluation of retinol, alpha-tocopherol and C-reactive protein at least once during the follow up. Serum concentration of alpha-tocopherol decreased significantly during the therapy, but retinol and C-reactive protein concentrations remained unchanged. In conclusion, a significant correlation was observed between serum retinol and C-reactive protein. Serum alpha-tocopherol decreased significantly during the first month of combination therapy with cetuximab. Low retinol and high C-reactive protein concentrations were predictive of poor prognosis in this patient population.

Key Words alpha-tocopherol, C-reactive protein, cetuximab, colorectal carcinoma, retinol

In the past decade, drugs targeting the epidermal growth factor receptor (EGFR) have been introduced into the therapy of solid tumors. Significant activity has been demonstrated in clinical trials for both low-molecular-weight inhibitors and antibodies blocking EGFR. Cetuximab is a chimeric antibody against EGFR registered for the therapy of advanced colorectal carcinoma. In a prospective clinical trial, cetuximab alone or in combination with irinotecan has demonstrated significant clinical activity in patients with irinotecan-refractory metastatic colorectal carcinoma (1). Activity has also been demonstrated for cetuximab in combination with chemotherapy in the first line of treatment of metastatic colorectal carcinoma in patients with tumors not harboring the K-ras mutation (2, 3).

Cancer and anticancer therapy are associated with oxidative stress (4–6), and disorders of antioxidant balance may be involved in the toxicity of anticancer treatment. Vitamin E represents a major circulating antioxidant (7). The term vitamin E denotes several naturally occurring tocopherols and tocotrienols, but alpha-tocopherol is responsible for most vitamin E activity (7). Disorders of antioxidant balance involving vitamin E may be responsible for the toxicity associated with radiotherapy (8, 9), or chemotherapy (10), and, in earlier studies, a decrease of circulating alpha-tocopherol
concentrations has been observed during systemic chemotherapy (4, 5, 11, 12). Decrease of circulating retinol concentrations has also been reported in patients with advanced cancer, and the decrease of alpha-tocopherol and retinol in cancer patients correlated with laboratory parameters of systemic inflammatory response, e.g., C-reactive protein (13, 14). The information on parameters of oxidative stress or systemic inflammatory response in patients treated with cetuximab is limited.

The aim of the present study was to investigate the changes of serum retinol, alpha-tocopherol and C-reactive protein during the first month of treatment with cetuximab and combination chemotherapy.

PATIENTS AND METHODS

Twenty-five consecutive patients with metastatic colorectal carcinoma, 17 males and 8 females, aged (mean±SD) 60±13 (range 32–75) y were included in the study. Twenty-three patients were treated with the combination of cetuximab (loading dose 400 mg/m², subsequently 250 mg/m² weekly) followed by irinotecan (180 mg/m²), leucovorin (200 mg/m²), and 5-fluorouracil (400 mg/m² bolus and 1,200 mg/m² for 46 h) every 2 wk (15). One patient received a modification of this regimen and one patient with hyperbilirubinemia was treated with this regimen omitting irinotecan. All patients had been previously treated with oxaliplatin, and all but one patient had been pre-treated with an irinotecan-containing regimen. The protocol was approved by the institutional ethical committee, and the patients signed informed consent forms.

Blood samples were taken before the start of therapy and at subsequent weekly visits during the first month of treatment. Serum alpha-tocopherol and retinol were determined before and during the therapy by high performance liquid chromatography as described (16). Blood samples were drawn from a peripheral vein after an overnight fast. The samples were transferred immediately to the laboratory and centrifuged (1,600 × g, 10 min, 16 °C), and the serum was separated and 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 min, 4 °C). Subsequently, 2,500 μL of n-hexane was added to this mixture and extracted for 5 min by a vortex apparatus. After centrifugation (1,600 × g, 10 min, 0 °C), the aliquot (2,000 μL) of the clean extract was separated and evaporated under nitrogen (60 °C). The residue was dissolved in 400 μL methanol and analyzed by reversed-phase high performance liquid chromatography using the 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. Separation of alpha-tocopherol and retinol was performed using the Chromolith Performance RP-18e, 100×4.6 mm monolithic column (Merck, Darmstadt, Germany). As the mobile phase 100% methanol was used at the flow rate of 2.5 mL·min⁻¹ and column pressure of 3.3 MPa. The 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. C-reactive protein was determined using particle-enhanced immunoturbidimetric assay as commercially available on MODULAR analyzer (Hoffmann-La Roche, Basel, Switzerland).

Measurements before and during the treatment was compared by the Wilcoxon signed rank test. The correlations were examined by Spearman’s correlation coefficient (rₛ). The survival of patients according the serum concentrations of retinol, alpha-tocopherol, and C-reactive protein was compared, using the log-rank test, after dichotomization according the cutoff limits selected based on medians of respective parameters in the studied group. All statistical analyses were performed using NCSS software (Number Cruncher Statistical Systems, Kaysville, Utah, USA).

RESULTS

Significant correlation was observed at the first visit (before the start of treatment) between concentrations of retinol and C-reactive protein (rₛ = −0.54, p<0.01;
Table 1. Retinol, alpha-tocopherol and C-reactive protein during the first month of therapy with cetuximab.

<table>
<thead>
<tr>
<th>Visit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>22</td>
<td>21</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Time from the start of therapy (d)</td>
<td>1±1</td>
<td>8±6</td>
<td>15±5</td>
<td>22±6</td>
<td>27±3</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>1.2±0.6</td>
<td>1.3±0.5</td>
<td>1.2±0.4</td>
<td>1.3±0.5</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Alpha-tocopherol (μmol/L)</td>
<td>27.5±7.6</td>
<td>23.5±6.9</td>
<td>24.9±6.2</td>
<td>22.1±6.6</td>
<td>19.0±8.2</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>40.8±47.4</td>
<td>28.8±25.5</td>
<td>22.9±29.1</td>
<td>20.2±27.5</td>
<td>20.3±24.8</td>
</tr>
</tbody>
</table>

Shown is the mean±SD of the respective parameters. *p<0.001, †p<0.05.

Fig. 1). Low serum concentrations of retinol and high concentrations of C-reactive protein were predictive of poor prognosis. Median survival of a patient who had at the first visit serum retinol below 1.25 μmol/L (n=13) was 10 mo compared to 18 mo for 12 patients who had serum retinol equal or above 1.25 μmol/L (p<0.05; Fig. 2). The median survival of patients who had serum C-reactive protein below 24 mg/L (n=12) was significantly longer compared to patients with C-reactive protein levels equal or above 24 mg/L (18 vs. 7 mo, p<0.05). No difference in survival was observed in patients with alpha-tocopherol concentration equal or above 26 μmol/L (n=12) versus below (11 mo vs. 13 mo; not significant).

Twenty-two patients had evaluation of retinol, alpha-tocopherol and C-reactive protein at least once during the follow up. The samples were obtained at approximately weekly intervals, usually immediately before weekly cetuximab administration. The mean (±SD) of subsequent measurements after baseline in these 22 patients was 3±1 (range 1–4). Fewer measurements were obtained in many patients because of technical reasons, postponement of chemotherapy visits, declining general condition of the patient, or because the patient started taking vitamin supplements. The patients had no major surgical procedure during the month preceding the start of therapy. In 5 patients, a central catheter with a subcutaneous port system was introduced into the subclavian vein immediately prior to the start of treatment. Skin rash accompanied the administration of the therapy in 19 patients. One patient had an infusion reaction, but other side-effects, including nausea, diarrhea or leucopenia were mild. During the study period fever and suspected catheter infection was observed only in one patient. Serum concentration of alpha-tocopherol decreased significantly during the therapy, but changes in the concentrations of retinol and C-reactive protein did not reach statistical significance (Table 1).

DISCUSSION

In the present study, a significant inverse correlation was observed between serum C-reactive protein and retinol concentrations. Despite the limited number of subjects investigated, high C-reactive protein and low retinol were predictors of poor prognosis. This is in agreement with other reports demonstrating prognostic significance of acute phase response in patients with metastatic colorectal carcinoma (17). The present study extends the association between acute phase response and prognosis to patients treated with combination chemotherapy and cetuximab, predominantly in the third or higher line of therapy.

Low circulating retinol concentration in patients with metastatic colorectal cancer is the result of acute phase response rather than a nutritional deficit. Circulating concentrations of alpha-tocopherol and retinol are known to be significantly decreased in patients with advanced cancer (13, 18). Retinol metabolism is associated with acute phase response as the key proteins responsible for retinol transport in the circulation, retinol binding protein and transthyretin, are negative acute phase proteins. Both retinol binding protein and transthyretin decrease after the administration of interleukin-6 (19). Consequently, retinol decreases as part of the acute phase response (20, 21). A negative correlation between C-reactive protein or other acute phase proteins and retinol has been documented in cancer patients (13, 14) as well as in patients with other inflammatory disorders associated with acute phase response (22). A correlation between C-reactive protein and alpha-tocopherol has been noted in some (13, 23), but not in other reports (14). In the present study, no association was observed between alpha-tocopherol and acute phase response, and serum alpha-tocopherol was not predictive of prognosis, but a correlation between serum retinol, acute phase response and prognosis was evident.

All patients in the present study were treated with the combination of cetuximab and chemotherapy, and it is not possible to discern the effects of individual drugs administered. Administration of chemotherapy is associated with oxidative stress that could result in lower concentrations of circulating antioxidants. However, in a recent study we observed increased alpha-tocopherol and retinol concentrations during paclitaxel/carboplatin combination chemotherapy (24) and hypothesized that this increase is linked to the suppression of systemic inflammatory response by chemotherapy. On the other hand, administration of chemotherapy is associated with small bowel dysfunction, and disturbances of the small bowel function may be accompanied by low circulating concentrations of retinol or alpha-tocopherol (25–27). The present observation of decreased alpha-tocopherol during the combination therapy is in agreement with earlier reports of
decreased alpha-tocopherol concentrations during systemic chemotherapy that were associated with oxidative stress induced by the therapy (4, 5, 11, 12). Serum alpha-tocopherol was decreased throughout the observation period, but the decrease at visit 5 did not reach statistical significance because of the smaller number of patients investigated.

It may be speculated that the decrease of serum alpha-tocopherol observed during cetuximab therapy may play a role in the toxicity of this drug. Skin toxicity is the most important side effect of treatment with cetuximab as well as with the low molecular weight EGFR inhibitors. Skin toxicity of EGFR inhibitors usually presents as acneiform eruptions involving seborrheic areas of the skin of the head, trunk or extremities (28), including periorbital skin or eyelids (29). In patients with metastatic colorectal carcinoma skin toxicity is a predictor of cetuximab efficacy, with patients experiencing skin toxicity having a significantly higher response rate compared to patients with no skin side effects (1). In an experimental study, epidermal growth factor has been shown to affect the expression of alphatocopherol transfer protein (30), but the exact mechanism of the decrease of alpha-tocopherol in patients treated with cetuximab remains to be determined. Alpha-tocopherol is a major skin antioxidant (31) that is secreted through the sebaceous glands, and high concentrations are observed in the outer skin layers (32). Decrease in vitamin E has also been linked by earlier investigations to skin disorders (31). Lipid peroxidation is thought to play an important role in the pathogenesis of acne (33). Further studies on a larger cohort of patients treated with anti-EGFR agents should address the association between skin toxicity and changes in vitamin E levels as well as the possibility of intervention by systemic or topical administration of this vitamin. The changes in markers of oxidative stress, e.g. thiobarbituric acid reactive substances, during the therapy depends on lipid oxidation (4). Vitamin E represents the major circulating antioxidant (7). The administration of antioxidants, including vitamin E, may delay atherosclerosis (36). In patients presenting for second or third line chemotherapy of metastatic colorectal carcinoma, the long-term effect of therapy on the risk of cardiovascular disease is of little concern. With the expanding use of cetuximab and chemotherapy in the first line setting and with improving long-term survival, however, changes affecting cardiovascular risk could be of greater importance.

In conclusion, a significant correlation was observed between serum retinol and C-reactive protein. Serum alpha-tocopherol decreased significantly during the first month of combination therapy with cetuximab. Low retinol and high C-reactive protein concentrations were predictive of poor prognosis in this patient population.

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REFERENCES


