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Effect of etoposide (VP16-213) on lipid peroxidation and antioxidant status in a high-dose radiochemotherapy regimen

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Summary. A total of 13 patients receiving bone marrow transplants (BMT) for treatment of different haematological diseases were investigated. Conditioning therapy preceding BMT consisted of fractionated total-body irradiation (12 Gy) and high-dose chemotherapy with cyclophosphamide (2 ± 60 mg/kg). Patients stratified to be at high risk for relapse (6/13) were additionally treated with etoposide (30 mg/kg). Plasma concentrations of absolute and lipid-standardized antioxidants (α-tocopherol and β-carotene) decreased following conditioning therapy, presumably as the result of an enhanced breakdown of these antioxidants. Etoposide treatment did not amplify the loss of essential anti-oxidants but significantly increased lipid hydroperoxide concentrations in serum. We suggest that the abnormal generation of lipid hydroperoxides is the result of free radical formation.

Introduction

Conditioning therapy preceding bone marrow transplantation (BMT) generally consists of total-body irradiation and high-dose chemotherapy with one or more antineoplastic agents such as cyclophosphamide and etoposide (VP-16). Such therapy places an enormous strain on tissues and metabolism, nearly reaching the tolerable limit [3]. A variety of experimental results suggest that peroxidative processes may occur during conditioning therapy and may be involved in early and post-transplant toxicity:

1. Cyclophosphamide depletes hepatic glutathione [10] and may thus contribute to oxidative tissue damage. A side effect of VP-16 is hepatotoxicity, which may be caused by glutathione depletion, as previously observed in mice [11].

2. Lipid peroxidation has been suggested to be one of the main causes of ionizing radiation damage. Indeed, loss of essential antioxidants has been observed in patients given cyclophosphamide alone or in combination with total-body irradiation [7]. Furthermore, there is evidence that in patients treated with total-body irradiation plus antineoplastic agents, lipid hydroperoxide generation is amplified [6]. This could be of particular interest for conditioning regimens including VP-16, since van Maanen et al. [12–14] have reported that this drug not only inhibits topoisomerase II but also possibly inactivates DNA via a semi-quinone radical and, thus, may amplify peroxidative tissue damage. On the other hand, VP-16 can inhibit basal and daunomycin-promoted microsomal lipid peroxidation [16, 17].

To investigate whether VP-16 exhibits an amplifying or attenuating effect on lipid peroxidation and the loss of antioxidants in vivo, we measured the lipid hydroperoxide production and the status of essential antioxidants before, during and after BMT.

Patients and methods

A total of 13 patients with acute leukemia, chronic granulocytic leukemia, myelodysplastic syndrome and severe aplastic anemia were studied. All patients were treated with fractionated total-body irradiation (12 Gy: 2 Gy twice daily for 3 days starting 7 days before BMT (day −7 to day −5). The day of BMT is numbered day O. The patients were divided into two groups: patients in group E^- were given cyclophosphamide (60 mg/kg for 2 days) starting 4 days before BMT (day −4 and day −3). Patients in group E^+ were additonally treated with VP-16 4 days before BMT (30 mg/kg on day −4).

Parenteral nutrition beginning on day −8 consisted of 500 ml 10% lipid emulsion daily (containing mainly oleum sojae fraction with about 26 g linoleic acid and about 4 mg D-α-tocopherol). Additionally, 5 mg a-tocopherol acetate was given i. v. in a multi-vitamin preparation. Blood was taken three times: 8 days before BMT (day −8), on the day of BMT (day 0) and 12 days after BMT (day +12).

α-Tocopherol and β-carotene concentrations in plasma and red blood cell membranes were investigated as previously described [7, 19]. Cholesterol measurement was done with a photometric test kit using the CHOD-PAP method (Merck; Darmstadt, FRG, No. 14366). Lipid hydroperoxide serum concentrations were determined by the CHOD-iodide method (Merck; Darmstadt, FRG, No. 14106) [8]. Statistical analyses were carried out using the SPSS computing package.

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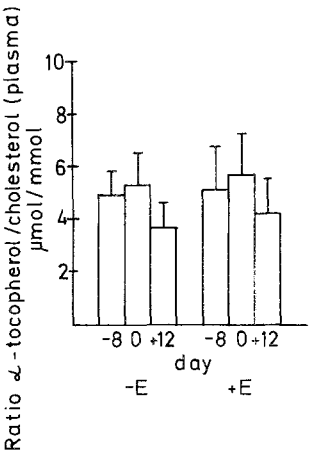

Fig. 1. Molar ratio of α-tocopherol/cholesterol in plasma before conditioning therapy (*day −8*), after conditioning therapy on the day of bone marrow transplantation (*day 0*), and 12 days after bone marrow transplantatioon (*day +12*) (mean ± SD). During the period investigated, 9 mg α-tocopherol was supplemented i. v. Statistical calculations were done by analysis of variance: time course: significant change, $P = 0.013$; E^+ vs E^-, not significant

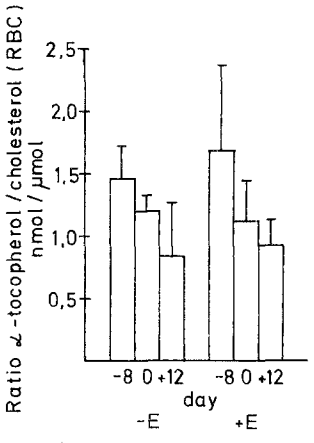

Fig. 2. Molar ratio of alpha-tocopherol/cholesterol in red blood cell membranes. For further explanations see Fig. 1. analysis of variance: time course: significant change, $P < 0.0001$; E^+ vs E^-, not significant

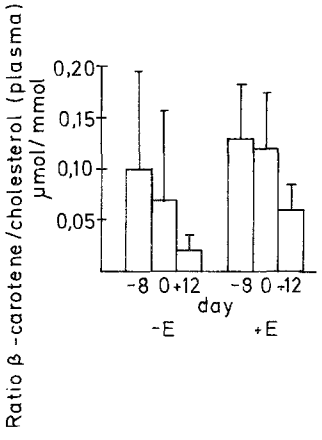

Fig. 3. Molar ratio of β-carotene/cholesterol in plasma. For further explanations see Fig. 1. Analysis of variance: time course: significant change, $P = 0.022$; E^+ vs E^- not significant

Results

α-Tocopherol

α-Tocopherol levels were expressed as lipid-standardized values (α-tocopherol/cholesterol), as previously suggested by Gey et al. [9]. The ratio dropped significantly between day −8 and day +12 ($P = 0.013$, analysis of variance) (Fig. 1). However, a comparison of both therapy modalities revealed no differences (analysis of variance for therapy and two-way interactions). Corresponding to the plasma concentrations, lipid-standardized α-tocopherol levels in red blood cell membranes also dropped between day −8 and day +12 ($P < 0.0001$, analysis of variance) (Fig. 2). A comparison of both therapy modalities revealed no difference (analysis of variance for therapy and two-way interactions).

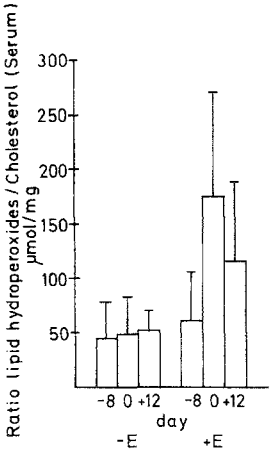

Fig. 4. Ratio of lipid hydroperoxides/cholesterol in serum. Analysis of variance shows a significant increase in the time-course study ($P = 0.017$) and a significant difference between both groups (E^+ vs E^-, $P = 0.003$). The significant increase in group E^+ is supported by calculations using the *t*-test for paired values ($P = 0.013$), whereas within group E^- there were no significant differences in time-course

β-Carotene

β-Carotene concentrations in plasma (P = 0.02; data not shown) and the ratio of β-carotene/cholesterol in plasma ($P = 0.022$; Fig. 3) decreased between day −8 and day +12 (analysis of variance). There were no differences between the two therapy modalities in calculations of the main effects and the two-way interactions.

Lipid hydroperoxides

Since lipid hydroperoxides in serum are mainly transported in the cholesterol-rich lipoprotein fraction, their concentrations were expressed as lipid hydroperoxides/cholesterol. Analysis of variance revealed significant differences between the two therapy modalities ($P = 0.003$) and the time course ($P = 0.017$). Calculation by the two-way interaction analysis resulted in a significant difference between therapy modalities and time-course changes ($P = 0.03$) (Fig. 4). The increase in lipid hydroperoxide concentration following conditioning therapy was apparent in the group treated with VP-16 (E^+). This significant increase was supported by calculations using the paired *t*-test ($P = 0.013$).

Discussion

The results demonstrate that a-tocopherol and β-carotene concentrations in plasma and red blood cell membranes could not be maintained at their initial status. Concentrations of α-tocopherol decreased during and after conditioning therapy, although it was supplemented with a dose (about 9 mg/day) recommended by the United States Food and Drug Administration (recommended dietary allowance: men, 10 mg/day; women, 8 mg/day) [15]. The decrease in α-tocopherol and β-carotene may be the result of an enhanced breakdown caused by the conditioning therapy. Exhaustion of β-carotene may additionally be an effect of the termination of the oral β-carotene supply at the beginning of the conditioning therapy.

Interestingly, two biological antioxidants were lost that differ in chemical structure, compartmentation and function. α-Tocopherol is the major lipid-soluble, chain-break-

ing antioxidant in human blood and tissue membranes [2], is effective at high oxygen concentrations and may be associated with most lipophilic cell structures. β-Carotene specifically complements the action of α-tocopherol at low oxygen concentrations [1]. Loss of α-tocopherol in combination with a markedly reduced β-carotene concentration might actually render organs much more susceptible to free-radical-induced tissue damage. This could be of particular interest in regimens including VP-16, since this drug may act by redox cycling, including free radical formation [12, 14].

Interestingly, VP-16 is more cytotoxic to normally oxygenated tumor cells than to hypoxic cells [18]. These in vitro findings support the present results, which showed an amplified generation of lipid hydroperoxides in patients treated with high-dose VP-16. The in vitro attenuation of lipid peroxidation in hepatic microsomes [16] is not a conflicting event, since this divergence between in vitro and in vivo lipid peroxidation is well known (e.g., phenylhydrazine [4, 5]) and may be attributed to a drug-dependent inactivation of enzymatic microsomal lipid peroxidation in vitro.

On the basis of these results, we propose intervention studies investigating the effect of high-dose β-carotene and a-tocopherol administration on the toxicity of intensive cancer chemotherapy protocols, particularly in regimens including etoposide.

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