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DECREASED ESSENTIAL ANTIOXIDANTS AND INCREASED LIPID HYDROPEROXIDES FOLLOWING HIGH-DOSE RADIOCHEMOTHERAPY

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The blood from 19 patients having bone marrow transplantation was examined for the essential antioxidants alpha-tocopherol and beta-carotene as well as lipid hydroperoxides before, at and after bone marrow transplantation (BMT). Conditioning therapy, preceding BMT in order to achieve marrow ablation and immunosuppression, consists of high-dose chemotherapy which is mostly combined with total body irradiation (TBI). In order to see a possible difference between patients with and without additional TBI, we divided the patients up into two groups; patients receiving TBI (RT^+) and patients without TBI (RT^-). All patients required total parenteral nutrition beginning one week prior to BMT.

After conditioning therapy plasma levels of absolute and lipid-standardized alpha-tocopherol and beta-carotene decreased in both groups, presumably as a result of an enhanced breakdown of these antioxidants. The loss of these lipid-soluble antioxidants has to be considered as a possible cause for early post-transplant toxicity. Lipid hydroperoxides increase significantly in the group of patients with additional TBI, whereas the other group, receiving no additional TBI, showed no significant change.

We suggest high-dose supplementation of essential antioxidants for patients undergoing BMT.

KEY WORDS: Lipid hydroperoxides, bone marrow transplantation, alpha-tocopherol, beta-carotene, radiation therapy, oxidative tissue damage.

INTRODUCTION

Conditioning regimens preceding bone marrow transplantation (BMT) consist of high-dose chemotherapy, generally combined with total body irradiation (TBI). These regimens approach the limit of tolerance for several tissues.[1] Lipid peroxidation has been suggested as one of the main causes of radiation damage.[2,3] Cyclophosphamide, which is frequently used in conditioning chemotherapy, depletes hepatic glutathione, thus potentially initiating peroxidative processes.[1,4] So toxicity of conditioning regimens may result in part from radical-mediated tissue damage.

Therefore, we measured lipid hydroperoxides (LOOH) in blood and investigated the status of essential antioxidants such as alpha-tocopherol (toc) and beta-carotene (car), which may be involved in the pathogenesis of acute and delayed post-transplant complications.

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PATIENTS AND METHODS

19 patients (10 male, 9 female, range 11–43 years, median 23) with either acute leukemias, chronic granulocytic leukemia, myelodysplastic syndrome, severe aplastic anemia (SAA) or neuroblastoma were studied.

One patient, suffering from SAA was examined again 4 months later during a course of a second BMT consecutive to a graft failure.

Patients were treated with different conditioning therapies: 4 patients only got cyclophosphamide (CP), 6 patients got CP and TBI, 7 patients got CP, TBI and etoposide (etop), 1 patient got CP and melphalan and 1 patient received TBI and melphalan. The day of bone marrow transplantation was numbered as "day 0". Cyclophosphamide was given on 2 consecutive days beginning day −4 (2 × 60 mg/kg body weight). Etoposide was given on day −4 (1 × 30 mg/kg body weight). Total body irradiation (12 Gy; lung shielding at 10 Gy) was fractionated (3 days twice daily 2 Gy), beginning on day −7. Melphalan was given on day −3 (180 mg/m^2).

The supportive nutritional therapy consisted of 500 ml lipid emulsion 10% daily (Intralipid®), which contains mainly Oleum sojae fraction with about 26 g linoleic acid and about 4 mg D-alpha-tocopherol.[5] Additionally, 5 mg DL-alpha-tocopherol acetate was given i.v. within multivitamin preparations (Multibionta® and Soluvit® with RDA doses of vitamins: thiamine, riboflavine, nicotinamide, pyridoxal, pantothenic acid, ascorbic acid, biotin, folic acid, cyancobalamin, retinol). Infusion of lipid emulsion and vitamins started on day −7 i.v. and was continued for 4 to 6 weeks after BMT. Patients received no exogenous carotenoids.

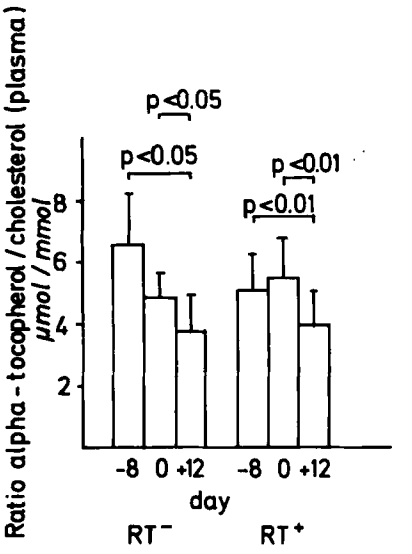

FIGURE 1 Molar ratio alpha-tocopherol/cholesterol in plasma. Values before conditioning therapy (day −8), after conditioning therapy and at day of bone marrow transplantation (day 0) and 12 days after bone marrow transplantation (day + 12). Patients were divided up into two groups; one group receiving no total body irradiation (RT$^-$), the other group receiving total body irradiation (RT$^+$). During the investigated period 9 mg alpha-tocopherol were supplemented intravenously. Mean ± S.D. Statistical calculations were performed using Student's t-test for paired values.

To investigate a possible difference between patients receiving TBI (RT^+) and others who did not (RT^-), we divided the patients up into two groups.

Cholesterol in serum was measured with a photometric test (Fa. Merck, FRG, Nr. 14366, CHOD-PAP-method), LOOH was measured in serum using the CHOD-Jodide-method (Fa. Merck, FRG, Nr. 14106). Alpha-tocopherol and beta-carotene were determined as previously described.[15]

Statistical analysis was performed by using the t-test for paired values. Analysis of variance was calculated by using the SPSS computing package.

RESULTS

Alpha-Tocopherol

Measurement of alpha-tocopherol (toc) was decreased in both groups. According to the proposal to express the vitamin E status as toc/cholesterol ratio,[6] we calculated the molar ratio (Figure 1), which decreased in both groups significantly (RT^-: day -8, $6.58 \pm 1{:}85\,\mu$mol/mmol; day 0, $4.83 \pm 0.79\,\mu$mol/mmol; day $+12$, $3.75 \pm 1.17\,\mu$mol/mmol; RT^+: day -8, $5.06 \pm 1.2\,\mu$mol/mmol; day 0, $5.43 \pm 1.36\,\mu$mol/mmol; and $3.91 \pm 1.11\,\mu$mol/mmol, day $+12$) after conditioning therapy. Tocopherol in red blood cell membranes showed a corresponding drop. Although there is no significant difference between both groups, which is shown by analysis of variance ($p = 0.6$) and in 2-way interaction ($p = 0.056$), the time course change, as calculated by an analysis of variance, is significant ($p < 0.001$).

Beta-Carotene

The ratio beta-carotene/cholesterol in plasma of patients receiving TBI (RT^+) decreased significantly ($p < 0.01$) during conditioning therapy (Figure 2) from $0.11 \pm 0.78\,\mu$mol/mmol (day -8) to 0.04 ± 0.03 (day $+12$). Patients without receiving TBI (RT^-) showed no significant decrease of this ratio. Analysis of variance

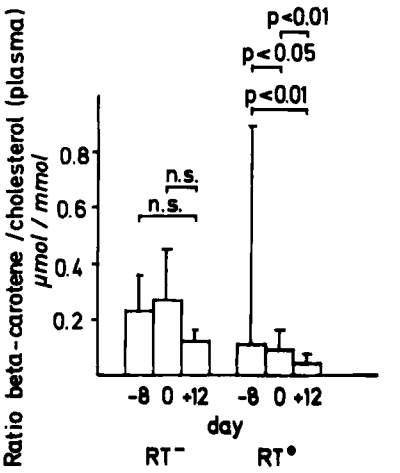

FIGURE 2 Molar ratio beta-carotene/cholesterol in plasma. For further explanation see Figure 1.

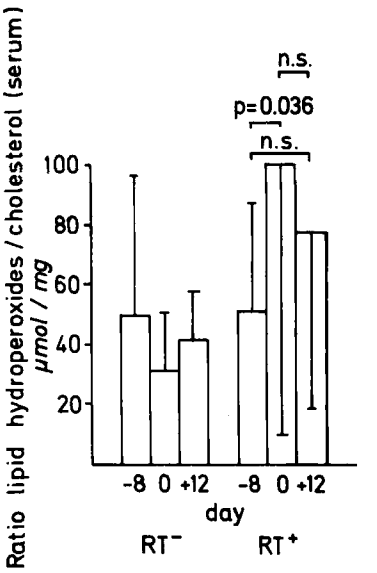

FIGURE 3 Ratio lipid hydroperoxides/cholesterol in serum. For further explanation see Figure 1.

showed a difference between both groups ($p < 0.005$). It is remarkable, that patients receiving TBI had less beta-carotene before starting conditioning therapy (RT^-: 0.23 ± 0.13 µmol/mmol on day -8 in comparison with group RT^+. This may be due to the chemotherapy previously applicated to the RT^+ group.

Lipid Hydroperoxides

Since LOOH are mainly carried in cholesterol rich lipoprotein fractions, we calculated the molar ratio between LOOH and cholesterol in plasma (Figure 3). Interestingly, a remarkable difference between both groups (RT^- respectively RT^+) could be seen. The RT^- group showed no significant change in LOOH ratio during conditioning therapy and after BMT; on the contrary, the RT^+ group showed an increase of LOOH of about 100% from day -8 to day 0. This increase was significant ($p = 0.036$). An analysis of variance showed this difference between RT^- and RT^+ group to be significant ($p < 0.05$).

DISCUSSION

The results show a considerable change in the status of the investigated antioxidants.

Although toc was supplemented in an amount of 9 mg/day, which follows the recommended dietary allowance (RDA: women 8 mg/day, men 10 mg/day),[7] the consumption seems not to be covered by this level of supplement. Since carotene (car) is not considered as a vitamin, no RDA exists. Diplock[8] suggested that the present RDA of vitamin E will prove to be too low and that there are cogent reasons for the

recognitition of beta-carotene as a vitamin in its own right. Our results support this suggestion in respect of special situations appearing with an enhanced requirement for antioxidants. Patients undergoing highly toxic cancer treatment, including chemotherapy, radiotherapy and receiving parenteral nutrition with their accumulating deleterious effects[9,5] seem to need a particular recommendation for the supplementation of antioxidants.

The loss of antioxidants in patients undergoing conditioning therapy may be due to various influences. As previously mentioned, cyclophosphamide, used in conditioning therapy, depletes hepatic glutathione and does potentially initiate peroxidative processes. TBI as well has been suggested to support these processes by lipid peroxidation.[2,3] The massive infusion of polyunsaturated fatty acids (about 26 g linoleic acid daily) may render the patients most susceptible to oxidative damage. Even under normal conditions 0.6 mg alpha-tocopherol is recommended per g linoleic acid consumed. Since beta-carotene is not included in the parenteral nutrition, its loss may partly be due to the termination of beta-carotene input one week prior to bone marrow transplantation.

Aiming at the chemical structure, compartmentation and function of the two investigated antioxidants, differences are seen. Alpha-tocopherol is the major lipid-soluble, chain-breaking antioxidant in human blood and tissue membranes,[10] which is effective at high oxygen concentrations and may be associated with most lipophilic cell structures. Beta-carotene complements the action of alpha-tocopherol specifically at low oxygen concentrations.[11] Loss of alpha-tocopherol in combination with a markedly reduced beta-carotene level might actually render organs much more susceptible to free radical induced tissue damage.

Contrary to the expected increased loss of antioxidants due to TBI, all conditioning regimens, with and without TBI, led to comparable losses of antioxidants.

Apparently, TBI does not intensify the exhaustion of lipid-soluble antioxidants. However, measurement of LOOH shows a significant elevation of LOOH in patients who received TBI. Different possible tissue damages appearing after conditioning therapy might result from an increased susceptibility due to a decreased antioxidant level.

The lung with its particular high oxygen tension might be an example,[12] since after BMT an interstitial pneumonia often occurs. Furthermore the life quality is affected after conditioning therapy by oral and intestinal mucositis, which may improve by a supplementation of antioxidants.[13]

Conceivably a specific clinical need for a high-dose antioxidant supplementation would be graft-versus-host reactions.

Activated granulocytes appearing during haematopoetic reconstitution and graft-versus-host reaction may serve as a source of oxygen radicals.

On the basis of these results we propose intervention studies investigating the effect of high-dose beta-carotene and alpha-tocopherol administration on the toxicity of intensive cancer chemotherapy protocols.

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