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INFLUENCE OF VITAMIN E ON OXIDATIVE STRESS PARAMETERS IN LIVER HOMOGENATES OF MICE TREATED WITH CYCHLOMIN

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ABSTRACT

The clinical efficacy of the antitumor drug 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (cychlomin, CCNU) is limited, as it cause various toxic side effects. In the present study we explored the alteration in oxidative stress parameters after administration of CCNU, and the influence of vitamin E on these parameters. The research was carried out with 48 mice separated in 8 groups. We found significantly increased MDA products and changes in SOD and CAT antioxidant activities in liver homogenates one hour after the administration of CCNU in both tumor and healthy mice compared to the controls. The combination of CCNU and vitamin E showed decreased levels of MDA and improvement of SOD and CAT activities in both tumor and healthy mice compared to CCNU administered alone. In conclusion, our results showed that CCNU therapy leads to excessive production of free radicals which leads to oxidative stress. Pretreatment with Vit. E could markedly suppress the hepatotoxic manifestations, observed in CCNU-treated mice.

Key words: oxidative stress, vitamin E, cytostatic therapy, lipid peroxidation, catalase.

INTRODUCTION

Normally a great number of reactive oxygen species (ROS) are produced in biological organisms as a result of biochemical oxireductive processes - superoxide anions (O_2), nitric oxide (NO), hydrogen peroxide (H_{2O2}), hydroxile radical (OH).

A low concentration of ROS is important for cell differentiation, proliferation, apoptosis, cell immunity, melanogenesis and other physiological processes [1]. Conversely high concentrations of ROS and their reactive derivates are dangerous for biological organisms, as they damage basic cell components [2]. Li pids are easily oxidizable which is why they are the main target for free radical damage through lipid peroxidation. Therefore lipid peroxidation is a foremost biomarker for ROS - induced damages [3].

Living organisms all contain complex antioxidative protective system which prevents the production of free radicals or limits their toxicity on cells. The system includes various enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and nonenzymatic substances - a-tocopherol (vitamin E), b-carotene, ascorbate (vitamin C), and glutathione (3]. The interruption of the balance between the processes producing ROS, and the antioxidative protective system results in oxidative stress, which may cause severe metabolic malfunctions and damage of biological macromolecules [3].

Main antineoplastic substances, used in chemotherapy are alkylating agents. Nitrosoureas, particularly the l-(2-

chloroethyl)-3-cyclohexyl-1 -nitrosourea (CCNU), show good therapeutic utility in various lymphomas, melanomas and brain tumors. Despite nitrosoureas' high in vivo activity, their clinical efficacy is limited, as they cause various side effects such as delayed, cumulative haematological toxicity, damage of the gastrointestinal tract, and delayed, cumulative dose-related hepatotoxicity [4]. Recent research showed that UV irradiation of lomustine (CCNU) leads to generation of ROS — ONOO‘ and ‘OH [5].

Therefore, the aim of the present study was to determine whether the antioxidant vitamíne E would prevent CCNU - induced oxidative liver damages in tumour bearing mice and thus provide some level of hepatoprotection.

MATERIALS AND METHODS

The study was carried out with 48 mice C57black, separated in groups - tumor and healthy. Tumor mice were inoculated with 10^s tumor cells from lymphoid leukemia L1210.

Preparation of tissue homogenates

Segments of the harvested livers were thoroughly rinsed with cold solution of 0.9% NaCl and dried out on filter paper. 0.5 g of the biomaterial was weighted out. The biomaterial was cut into small pieces and homogenized in ice-cold PBS buffer containing 1 mM EDTA-2Na, in proportion 1:10 (0.5 g: 5 ml). The homogenate was centrifuged at 3000 rpm for 15 min. and supernatant was used to measure malondialdehyde (MDA) levels and to determine enzyme activities.

Qualification of lipid peroxidation

Total amount of lipid peroxidation was estimated using the thiobarbituric acid (TBA) method, which measures the malondia-ldehyde (MDA) reactive products [6].

Determination of SOD and CAT in tissue homogenates

CuZn-SOD activity was determined as described by Sun et al [7]. The xanthine/ xanthine oxidase system was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazan, which is monitored at λ = 560 n m.

For measurement of CAT activity hydrogen peroxide was used as a substrate and the decrease in its concentration at 22°C in phosphate buffer was followed spectrophotometrically at 240 nm [8].

RESULTS

The results of lipid peroxidation are presented in Figure 1. We found significantly high MDA products in liver homogenates, isolated from tumor and healthy mice, one hour after the administration of CCNU, compared to controls (mean 1.478 µM vs 0.596µM $p<0.0001$ and 0.787µM vs 0.508µM, $p<0.01$). Tumor mice treated with CCNU, showed over 100% higher level of MDA products compared to healthy mice, injected with the same dose of CCNU. There was no significant increase in levels of MDA products, in healthy mice treated with Vit.E. Lower MDA levels were demonstrated in tumor mice, treated with Vit.E but there was no statistically difference between this group and mice treated only with CCNU (mean 1.140µM, $p>0.05$). We found that the combination of CCNU and vitamin E leads to slight decrease in level of MDA products in both tumor and healthy mice, compared to groups where CCNU is administered alone (mean 0.741 µM, $p>0.05$ and 1.275//M, $p>0.05$).

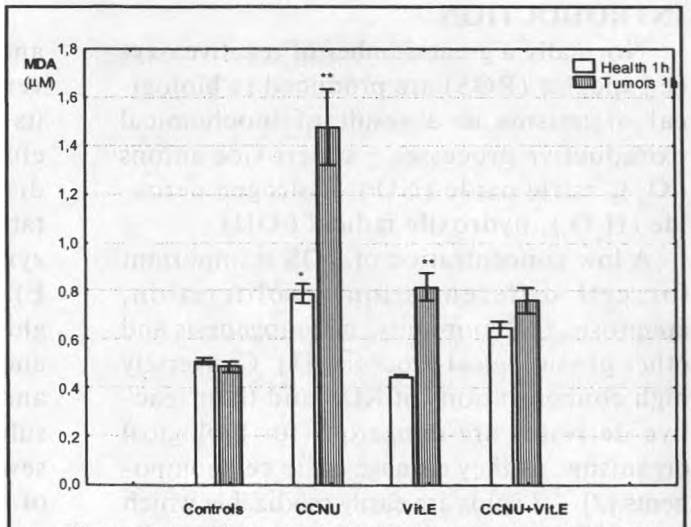

Figure 1. Lipid peroxidation in liver homogenates isolated from mice treated with CCNU, Vit. E, and their combinations. Values are expressed as mean ±SE. *p<0.05 *vs* healthy controls; **p<0.001 *vs* tumor controls.

The results of SOD activity in liver homogenates are presented in Figure 2. During the first hour SOD activity was significantly lower in liver homogenates from tumor mice compared to healthy mice (mean 5.294 U/gPr, and 5.23 U/gPr vs 13.216 U/gPr, $p<0.0001$). In contrast, one hour after the administration of CCNU we found increase SOD activity in both tumor and healthy mice, compared to controls (mean 19.756 U/gPr and 18.621 U/gPr, $p<0.0001$). Tumor mice treated with Vit.E alone, registered higher SOD activity than healthy controls (mean 24.453 U/gPr, $p<0.0001$). When CCNU was administered in combination with Vit E, there was no significant decrease in SOD activity, compared with groups treated only with CCNU (mean 20.149 U/gPr and 23.478 U/gPr, $p>0.05$).

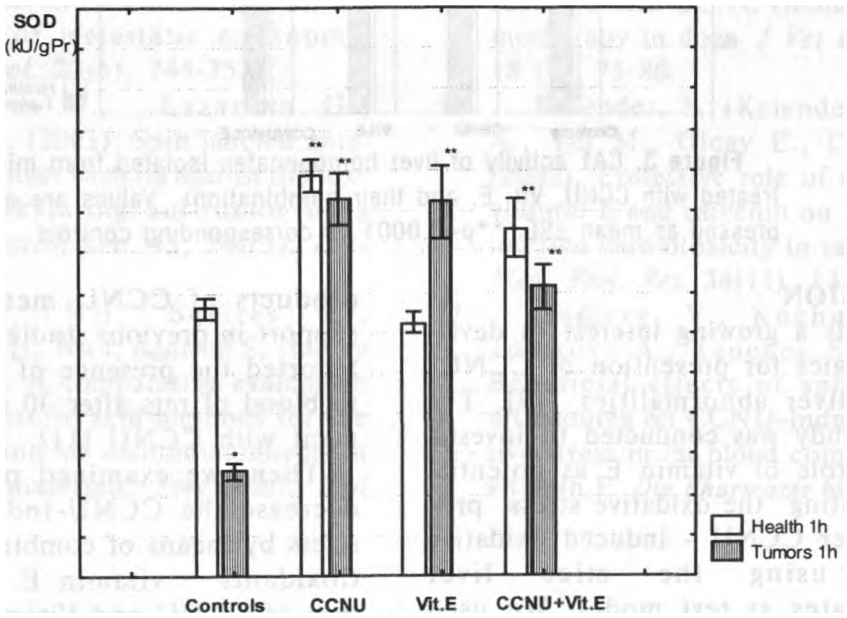

Figure 2. SOD activity of liver homogenates isolated from mice treated with CCNU, Vit. E, and their combinations. Values are expressed as mean ±SE. *$p<0.001$; **$p<0$ 0001 vs corresponding controls

The results of CAT activity in liver homogenates are presented in Figure 3. One hour after the administration of CCNU we found increased CAT activity in tumor and healthy mice, compared to controls (mean 47.254 U/gPr and 59.486 U/gPr and, $p<0.001$). There was a significantly increased CAT activity in liver homogenates, one hour after treatment with Vit.E (mean 66.596 U/gPr). When the combination of CCNU and Vit.E was administered in both tumor and healthy mice, we found a significant normalization of CAT activity, compared to groups with CCNU treatment without Vit.E (mean 36.952 U/gPr and 31.07825 U/gPr, $p<0.0001$).

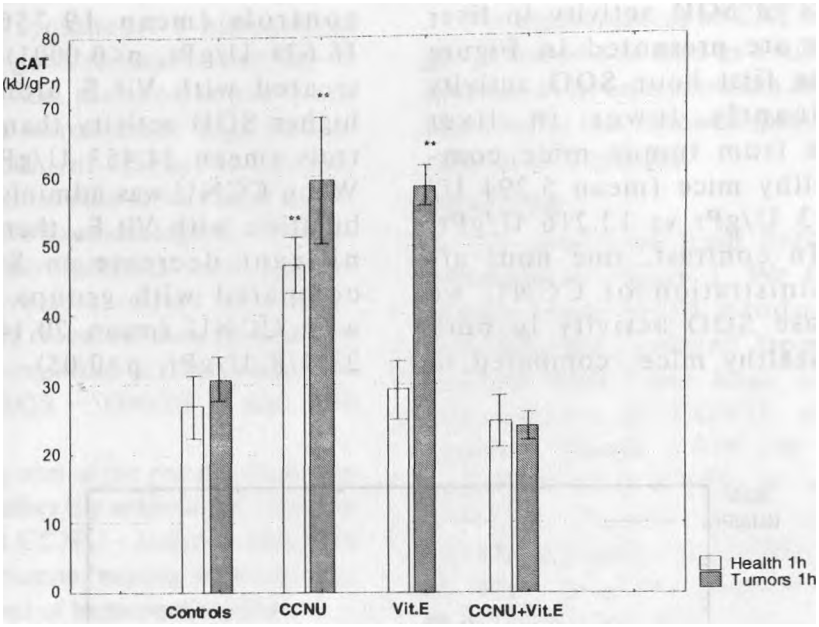

Figure 3. CAT activity of liver homogenates isolated from mice treated with CCNU, Vit. E, and their combinations. Values are expressed as mean +SE. **p<0.0001 vs corresponding controls.

DISCUSSION

There is a growing interest in devising strategies for prevention of CCNU-induced liver abnormalities 19]. The present study was conducted to investigate the role of vitamin E as potential for alleviating the oxidative stress produced after CCNU - induced oxidative injury, using the mice liver homogenates as test model. We used vitamin E since it influences the efficacy of many drugs currently used in cancer treatment [10].

We found that in 1 hour following treatment with CCNU, liver homogenates of both healthy and tumour-bearing mice had higher productions of lipid peroxidation, compared to controls and it was accompanied by increased activities of the antioxidant defence enzymes SOD and CAT. It is well known that increased production of lipid peroxides might be due to increased levels of ROS and could be connected with the presence of oxidative stress. This suggests increased risk for oxidative damages, due to increased generation of ROS -products of CCNU metabolism during the chemotherapy.

Augmented generation of toxic reactive oxygen species (ROS), which are products of CCNU metabolism, finds support in previous studies. We have also reported the presence of oxidative stress in blood of rats after 30 days oral treatment with CCNU [11].

Then, we examined possible way to decrease the CCNU-induced oxidative stress by means of combination with antioxidants — vitamin E. the combination of CCNU and Vitamin E, decrease MDA levels compared to groups where CCNU was administered alone. We found no significant changes in SOD activity but the activity of the antioxidant enzyme catalase was nearly normalized.

Stabilization of oxidative stress parameters after treatment with combination of CCNU and vitamin E may be due to the vitamin's protective effect on cell structures and another evidence for ROS-induced toxicity of CCNU. In view of these facts we can conclude that pretreatment with Vit. E can markedly suppress the hepatotoxic manifestations, observed in CCNU-treated mice.

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