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Original Contribution

INFLUENCE OF SPIN-LABELED ANTIOXIDANTS ON OXIDATIVE TOXICITY OF CYTOSTATIC THERAPY AND IONIZING RADIATION

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ABSTRACT

The aim of the present study is to explore the alteration in oxidative stress parameters after administration of some antitumor drugs, ionizing radiation and the influence of SLENU. The research was carried out with healthy and tumor mice C57 black. We found significantly high MDA and changes in SOD and CAT activity in liver homogenates, isolated from tumor controls and from tumor mice treated with antitumor drugs and radiation. The combination of SLENU and cytostatic drugs showed improvement in enzyme activities compared to mice treated with cytostatic drugs alone. In conclusion SLENU reduces oxidative injuries induced by antitumor therapy and ionizing radiation and thus provides hepatoprotection.

Key words: SLENU, MDA, SOD, CAT, antitumor drugs, ionizing radiation

INTRODUCTION

Normally a great number of reactive oxygen species (ROS) are produced in biological organisms as a result of biochemical oxireductive processes - superoxide anions ($.0_2$-), nitric oxide (NO), hydrogen peroxide (H_20_2), hydroxile radical (OH).

A low concentration of ROS is important for cell differentiation, proliferation, apoptosis, cell immunity, melanogenesis and other physiological processes [6]. Conversely high concentration of ROS and their reactive derivates are dangerous for biological organisms, as they damage basic cell components [4]. Lipids are highly 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 [5]. 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, and non-enzymatic substances - a-tocopherol (vitamin E), b-carotene, ascorbate (vitamin C), and glutathione [5]. 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 [5].

Numerous antineoplastic substances, used in chemotherapy exercise their cytostatic effect through free radical mechanism and they also induce oxidative stress. Bleomycin is a chemotherapy antibiotic which mechanism of action on DNA is similar to those of ionizing radiation - through generation of ROS, single and double-strand breakage of DNA is caused. Farmorubicin is an epimer of anthracycline chemotherapy drug - Adryamycin. Most likely, the mechanism of action of Anthracyclines is through inhibition of nuclear enzyme DNA-topoisomerase Π which results in protein-linked single and double-strand breakage of DNA. Excessive production of superoxide anions is related to the use of Farmorubicin. Cychlomin, Lomustine (CCNU) is a nitrosourea which refers to the group of alkylating agents and shows good therapeutic utility in various

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lymphomas, melanomas and solid tumors. Dispite nitrosoureas' high in vivo activity, their clinical efficacy is limited as they cause delayed dose-related hepatotoxicity (such as ascites, hyperbilirubinaemia, and thrombocytopenia) which sometimes leads to lethality [7]. The mechanism of Lomustines' toxic effect is through generation of superoxide anions. By EPR technique Gadjeva et al., have found that spin labeled nitrosoureas including SLENU exhibit high superoxide anion scavenging activity which was comparatively with that of some referent antioxidants as Vitamin C and Vitamin E [3]. Antioxidant activity of spin-labeled nitrosourea (SLENU) was proven in our laboratory.

Therefore the aim of the present study was to determine whether SLENU could reduce oxidative injuries induced by therapy with CCNU, Bleomycin, Farmorubin and ionizing radiation in liver of mice and thus provide some level of hepatoprotection. Markers of this effect were plasma level of MDA , SOD and CAT enzyme activities in liver of healthy and tumor-bearing mice.

MATERIALS AND METHODS

The study was carried out with mice C57black, separated in groups - healthy and tumor. Tumor mice were inoculated with 10^5 tumor cells from lymphoid leukemia.

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. 0.5g 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.

Quantification of lipid peroxidation. Total amount of lipid peroxidation was estimated using the thiobarbituric acid (TBA) method, which measures the malondialdehyde (MDA) reactive products [2].

Determination of SOD and CAT in tissue homogenates. CuZn-SOD activity was determined as described by Sun et al. [8]. The xanthine/xanthine oxidase system was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazan, which is monitored at $\lambda = 560$ nm. 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 [1].

RESULTS

The results of lipid peroxidation are presented in figure 1. We found significantly high MDA products in liver homogenates, isolated from tumor controls and from tumor mice treated with CCNU, Bleomycin, Farmorubicin and radiation (12 hours after administration) compared to the healthy controls (0.453+0.03 μM/gPr vs 0.595±0.02 μM/gPr, 0.973±0.16 μM/gPr, 0.78 l±0.03pM/gPr, 0.814±0.12 μM/gPr, 0.698±0.11 μM/gPr; $p<0.05$). Tumor mice treated with CCNU showed over 50% higher level of MDA products compared to healthy mice, treated with the same dose of CCNU (0.973±0.16pM/gPr vs 0.453±0.03gM/gPr, $p<0.05$). However liver homogenates prepared from mice treated with SLENU alone showed level of MDA products close to healthy controls (0.404+0.06μM/gPr vs 0.453±0.03μM/gPr).

The combination of Farmorubicin and SLENU showed slight decrease in levels of MDA products compared to cases of Farmorubicin administered alone (0.827±0.08μM/gPr vs 0.814±0.12μM/gPr). The combination of radiation treatment and SLENU led to a strong decrease in level of MDA products close to healthy controls, compared to radiation administered alone (0.543±0.07μM/gPr vs 0.698±0.11 μM/gPr, $p<0.05$). We found that the combination of CCNU and SLENU led to stronger decrease in level of MDA products compared to CCNU administered alone (0.509±0.05μM/gPr vs 0.973±0.16μM/gPr, $p<0.000l$).

The results of SOD activity in liver homogenates are presented in Figure 2. SOD activity was significantly higher in liver homogenates from tumor mice treated with CCNU, Bleomycin, Farmorubicin and radiation alone, compared to tumor control mice (10.971±1.23U/gPr, 11.584±0.44U/gPr, 14.246±2.27U/gPr, 6.713±0.93U/gPr vs 5.294±0.37U/gPr;$p<0.0001$). The combination of Bleomycin, Farmorubicin or radiation with SLENU showed higher SOD activity compared to tumor controls (11.925±2.31U/gPr, 11.432±1.12U/gPr and 10.698±1.40U/gPr vs 5.294+0.37U/gPr,$p<0.0001$). Moreover, tumor bearing mice treated with combination

of radiation and SLENU showed statistically stronger increase in SOD activity compared to mice treated with radiation alone (10.698± 1,40U/gPr vs 6.713±0.93U/gPr, p<0.0001). Therefore SLENU antagonizes to a great extent the effect of γ-irradiation. The combination of CCNU and SLENU decreased slightly SOD activity compared to tumor bearing mice treated with CCNU alone (9.523±0.56U/gPr vs 10.971 ±1.23U/gPr,p<0.05).

The results of CAT activity in liver homogenates are presented in Figure 3. CAT activity was significantly higher in tumor control mice compared to healthy controls (52.429±4.1 III/gPr vs 30.848±5.63U/gPr, p<0.05). Tumor mice treated with Bleomycin and those exposed to radiation showed highest CAT activity (53.132±3.91 U/gPr and 40.590±7.49U/gPr). The combination of Bleomycin and SLENU led to significantly lower CAT activity compared to Bleomycin administered alone (53.132±3.91 U/gPr vs 32.200±4.72U/gPr, p<0.0001). Tumor bearing mice treated with combination of Farmorubicin and SLENU showed lower CAT activity compared to tumor control mice (39.415±4.80U/gPr vs 52.429±4.11 U/gPr, p<0.05).

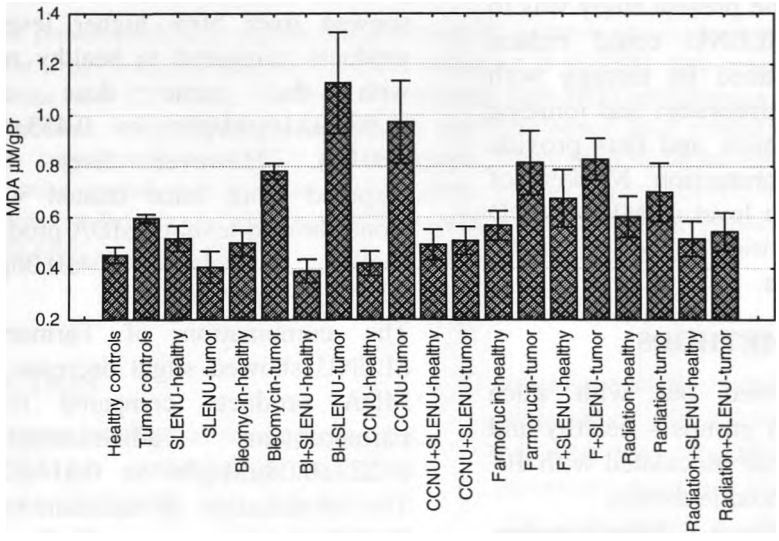

Figure 1. Lipid peroxidation in liver homogenates isolated from mice treated with SLENU,Bleomycin, CCNU.Farmorubicin,radiation and combinations with SLENU.

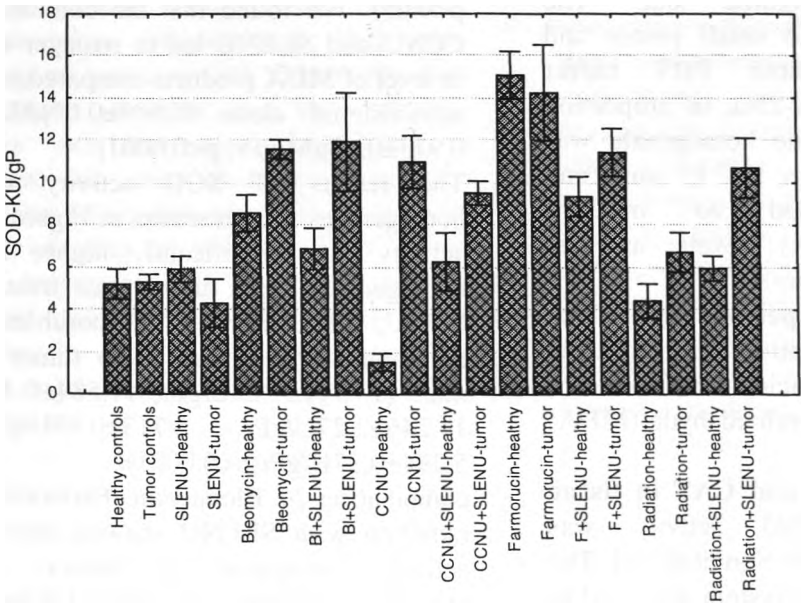

Figure 2. SOD activity of liver homogenates isolated from mice treated with SLENU, Bleomycin.CCNU,Farmorubicin,radiation and combinations with SLENU.

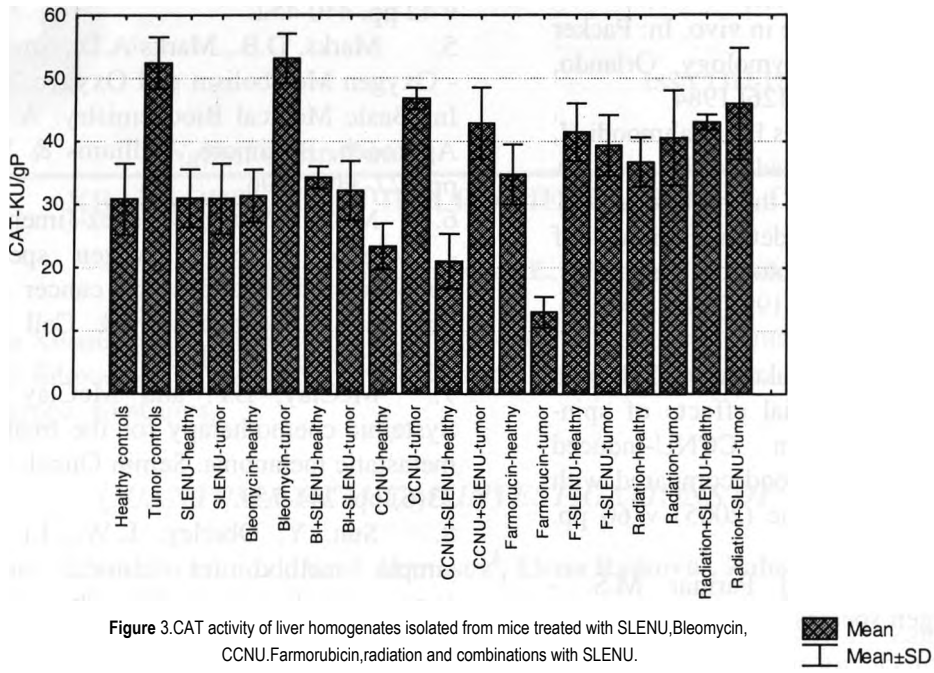

Figure 3.CAT activity of liver homogenates isolated from mice treated with SLENU,Bleomycin, CCNU.Farmorubicin,radiation and combinations with SLENU.

DISCUSSION

The present study was conducted to investigate the role of spin-labeled nitrosourea SLENU in moderating oxidative stress produced after administration of several chemotherapy drugs - Bleomycin, CCNU and Farmorubicin, and ionizing radiation, using mice liver homogenates as a test model.

We found that in 12 hours following treatment with Bleomycin, CCNU, Farmorubicin and radiation, liver homogenates of tumor-bearing mice had higher productions of lipid peroxidation, compared to controls and it was accompanied by increased activity of the antioxidant defense enzymes CAT and SOD. 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 chemotherapy drugs metabolism.

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 [3].

When SLENU is using along with chemotherapy drugs and radiation, it reduces lipid peroxidation and improves antioxidant enzymes CAT and SOD' activities in liver homogenates. Stabilization of oxidative stress parameters after treatment with combination of SLENU and chemotherapy drugs (especially CCNU) may be due to SLENU' protective effect on cell structures. In view of these facts we can conclude that pretreatment with SLENU can markedly suppress the hepatotoxic manifestations, observed in chemotherapy drugs-treated mice.

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