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Original Contribution

MITIGATION OF OXIDATIVE STRESS IN CYCLOPHOSPHAMIDE TREATED RENAL MODEL SYSTEM

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ABSTRACT

Endoxan (cyclophosphamide) is a cyclic propylene phosphamide ester of nitrogen mustard. Endoxan- main advantage of chemotherapy is complete penetration of the tissues, reaching the most widely spread malignant cells. Endoxan is a "transport form" and as such it has a selective tumour affinity. It consists of a carrier molecule, a blocking group to make it inactive during the transport stage, and the active nitrogen mustard group. It is inactive in vitro. Endoxan is used for active treatment of all neoplastic diseases of the reticulo-endothelial system, e.g. lymphomas, lymphosarcomas, reticular-sarcomas, Hodgkin's disease, chronic lymphatic leukaemias, multiple myelomas. Concentration of used Endoxan in our experiments is equivalent with mean therapeutic concentration used in chemotherapy of tumours. Endoxan was tested at renal supernatant and free enzyme model systems, for oxidative stress activity. The ability of Endoxan to interact with the superoxide radical, to influence their generation and probablity to change the levels of lipid peroxidation in model systems was investigated. The ability of Endoxane to affect Fe^{2+}-induced lipid peroxidation, estimated by the products from that reaction MDA are evaluating by spectrophotometry. Results of Fe^{2+}-induced lipid peroxidation in model systems ***in vitro*** show that Endoxan in investigated concentrations 10^{-4}; 10^{-5} M, have small but significant differences in MDA/TBA system for 10^{-4} M Endoxan. About superoxidedismutase activity (SOD), we obtained for the control 0.367 IU/mg protein, for Endoxane group, 0.433 IU/mg protein for 10^{-4} M,and for 10^{-5} M - 0.470 IU/mg protein as well. For more specific information about oxidative stress in renal model system caused by Endoxan, we use NBT test for evaluation of the endoxan activity in comparison with control group, the results show that the SpSI is 97 % for the 10^{-4} M Endoxan and 96 % for 10^{-5} M Endoxan. Due to its-oxidative tolerance, endoxan could be used in insertion in liposomes and this could impact endoxan tissue penetration.

Key words: endoxan, oxidative stress, model systems

INTRODUCTION

Endoxan is an alkylating agent used for the treatment of various types of cancer and is also used as a potent immunosuppressant but its administration has been associated with free radical mediated oxidative stress [1-3]. Endoxan evoked an increase of the level of the products of lipid peroxidaton and a decrease in the level of non-enzymatic antioxidants [3], such as reduced glutathione and vitamins C, E and A, as well as total antioxidant status [4, 9]. According some which is metabolised into active metabolites mainly in the liver, but its metabolites causes all that damages [1-4].

Some of researchers and clinicians are focused into development of new ways of administration of endoxane in order to decrease toxic and prooxidant effects of the drug on bladder testis plasma, aorta and other tissues [9].

The present study was designed to investigate the ability of endoxan to influences Fe^{2+}-induced lipid peroxidation in model system ***in vitro*** - renal supernatant system.

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MATERIAL AND METHODS

Model systems and tissue homogenates for in vitro study

Liposomal suspension. A liposomal suspension obtained from phospholipids of egg yolk extracted according to Folch [5] was used. After lyophilisation, the chloroform fraction was dissolved to 50 mM K-Na phosphate buffer pH 7.4 (PBS) to the final concentration of 5 mg lipid/ml.

Renal homogenate. Wistar rats (180-200g) were used. Kidneys were washed ***in situ*** with ice-cold 1.15% KC1. The homogenization was carried out in PBS at a ratio tissue: PBS = 1:3 (w/v). The homogenate was dissolved with PBS to contain lmg/ml protein.
After homogenisation of the homogenate we centrifuge (10 min 5000 rpm) and take out supernatant as a working media. All measurements were provided at renal supernatant.

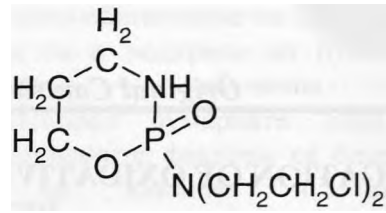
Registration of TBARS
The TBARS of LPO was measured in liposomal suspension and in renal supernatant after incubation for 20 min at 37 °C. Each sample included 1 ml supernatant and 0.8 ml PBS (or 1.8 ml liposomal suspension with concentration of 1 mg lipid/ml). The induction of LPO was initiated by adding of $FeCl_2$ to the samples. The complex was obtained by equimolar mixing of $FeCl_2$ to a final concentration of 1 mM. The TBARS generated in the system was determined according to Asakava and Matsushita [6]. Protein concentration was measured by Lowry et al [7J.
The activity of SOD in renal homogenate was determined by Geller and Winge (1984) method by reduction of NBT to formazan [10].

ALL REAGENTS OE ANALYTICAL GRADE WERE OBTAINED FROM ALDRICH CHEM. CO., HENKEL CO., MERCK, SIGMA CHEM. CO.

The drug was dissolved in PBS, pH 7.4. The final concentrations in the samples investigated are shown in the figure legends.

The statistic processing of the results is performed with the program for analysis MANOVA. The statistic differentiation of the results is determined by Bonferroni's test. The data are presented as value ± SD.

Structural formulae of Endoxan



RESULTS AND DISCUSSION

Often, the net therapeutic effect of the drugs is a combination of their specific and antioxidant effect. Figure 1 illustrates the effects of endoxan on the level of lipid peroxidation products such TBARS in renal supernantant. It was established that no significant changes in the content of TBARS in the group with or without endoxan in concentration range 10^{-4} - 10^{-5} M.

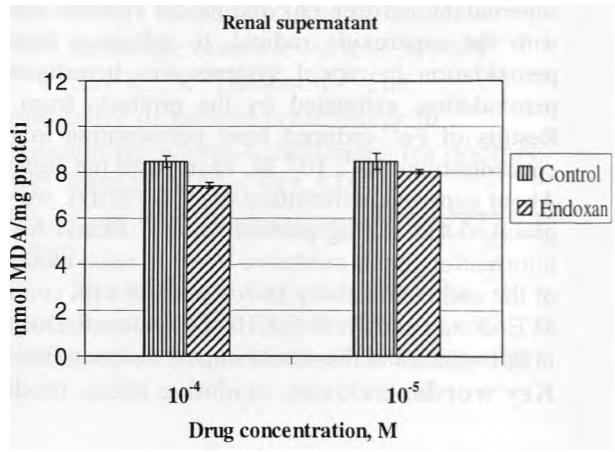


Figure 1. Effect of endoxan on the level of TBARS after Fe^{2+} - induced lipid proxidation in renal supernatant.

About superoxidedismutase activity (SOD), we obtained for the control 0.367 IU/mg protein, for

Endoxane group - 0.433 IU/mg protein for 10^{-4} M, and 0.470 IU/mg protein for 10^{-5} M Endoxan as well (Figure 2). Endoxan decreases on a small scale the activity of SOD in renal suspension.

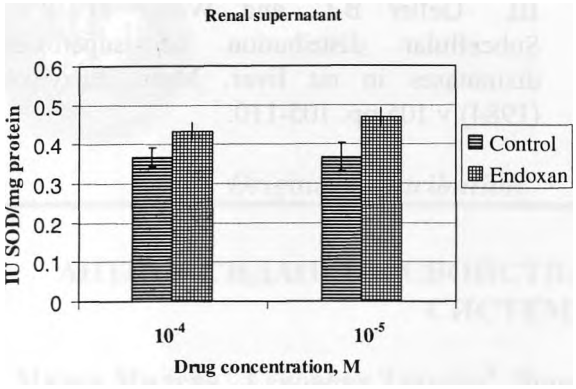


Figure 2. *Effect of endoxan on the level of SOD activity in renal supernatant.*

Our further experiments focused on the superoxide scavenging properties of Endoxan. We used a xanthine - xanthine oxidase system to generate superoxide, and a photometric method for registration of formazan (NBT-test). The samples were incubated at 37 °C and the amount of the formed formazan was measured by absorption at 560 nm. The time of incubation was selected so that the absorption for the controls was 0.2. The ratio of the absorption at 560 nm for the sample, containing endoxan versus the absorption of the control in percents is called spectrophotometric scavenger index (SpSI). As shown in Figure 3, endoxan does not show any superoxide scavenging properties in the systems used.

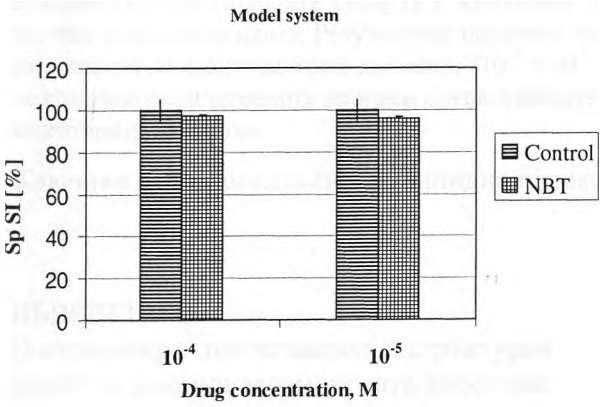


Figure 3. *SpSI of endoxan in xanthine - xanthine oxidase system for generation of superoxide*

In conclusions, the results obtained with the described experiments suggest that the observed med antioxidant effect of endoxan in vivo is not connected with its direct effect upon the Fe^{2+} - induced free radical processes in our model system. Probably this effect of endoxan is connected with its metabolism from the renal monooxygenases, leading to receiving of products with prooxidant effect.

Results obtained at the present study suggest that endoxan could be used in insertion in liposome due to endoxan-oxidative tolerance and this could impact endoxan tissue penetration.

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