Effects of endoxan on oxidative processes in model systems in vitro.

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ANTIOXIDANT PROPERTIES OF ENDOXAN IN RENAL MODEL SYSTEMS

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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. It is one of the most useful cytotoxics available today. 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. The cytotoxic action is presumably based on binding and inactivation of the cellular DNA. 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 in tumours, we suppose that at these levels of endoxan interact with renal cells and probably induce or inhibit new generation of superoxide free forms in same tissue. 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 probably to change the levels of lipid peroxidation (LPO) in model systems were investigated.

The ability of Endoxane to affect Fe^{2+}-induced lipid peroxidation, and products from that reaction MDA are evaluating by spectrophotometry. Results of our investigation on Fe^{2+}-induced lipid peroxidation in our 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 we have 7.43 nmol MDA/mg protein, for 10^{-5} M Endoxan we have 8.07 nmol MDA/mg protein. Values for the control samples which do not contain Endoxan are as follow: 8.48 nmol MDA/mg proteins. About Superoxidedismutase system (SOD), we obtained for the control 0.367 IU/mg protein, for Endoxane group we have for 10^{-4} M, 0.433 IU/mg protein, for 10^{-5} M, we have 0.470 IU/mg protein as well.

For more specific information about oxidative stress in renal model system caused by Endoxan, we use MBT test for evaluation of the endoxan activity in comparison with control group, the results show that we have 97 SpSI [%] for the 10^{-4} M Endoxan and 96 SpSI [%] for 10^{-5} M Endoxan. According that results that due to endoxan-oxidative tolerance, endoxan could be used in insertion in liposomes and this could impact endoxan tissue penetration.

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. Endoxan evoked an increase of the level of the products of lipid peroxidaton and a decrease in the level of non-enzymatic antioxidants, such as reduced glutathione and vitamins C, E and A, as well as total antioxidant status. According some authors endoxan is an inactive cytostatic which is metabolised into active metabolites mainly in the liver, but its metabolites causes all that damages.

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.

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.

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 was used. After lyophilisation, the chloroform fraction was dissolved in 50 mM K-Na phosphate buffer pH 7.4 (PBS) to a 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% KCl. The homogenization was carried out in PBS at a ratio tissue: PBS = 1:3 (w/v). The homogenate was dissolved with PBS to contain 1mg/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 ^{O}C. Each sample included 1 ml homogenate 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 Protein concentration was measured by Lowry *et al.*

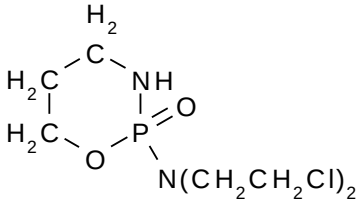
Content of the phospholipids was estimated by the method of Stewart.

All reagents of 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 multifactor analysis ANOVA. The statistic differentiation of the results is determined by Bonferroni's test. The data are presented as value ± SD.

Structural formulae of Endoxan



Results

Effect of endoxan on the level of TBARS in renal supernatant.

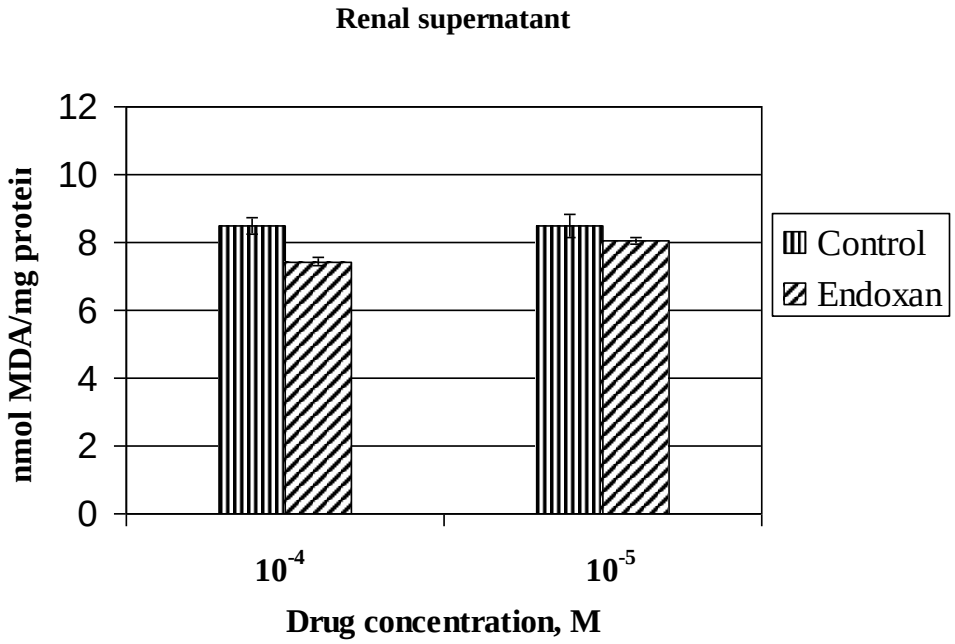


Figure 1 *illustrates the effects of endoxan on the level of lipid peroxidation products such MDA in renal supernantant*. It was established that no significant changes in the content of MDA in the group whit and without endoxan in concentration range 10^{-3} – 10^{-4} M.

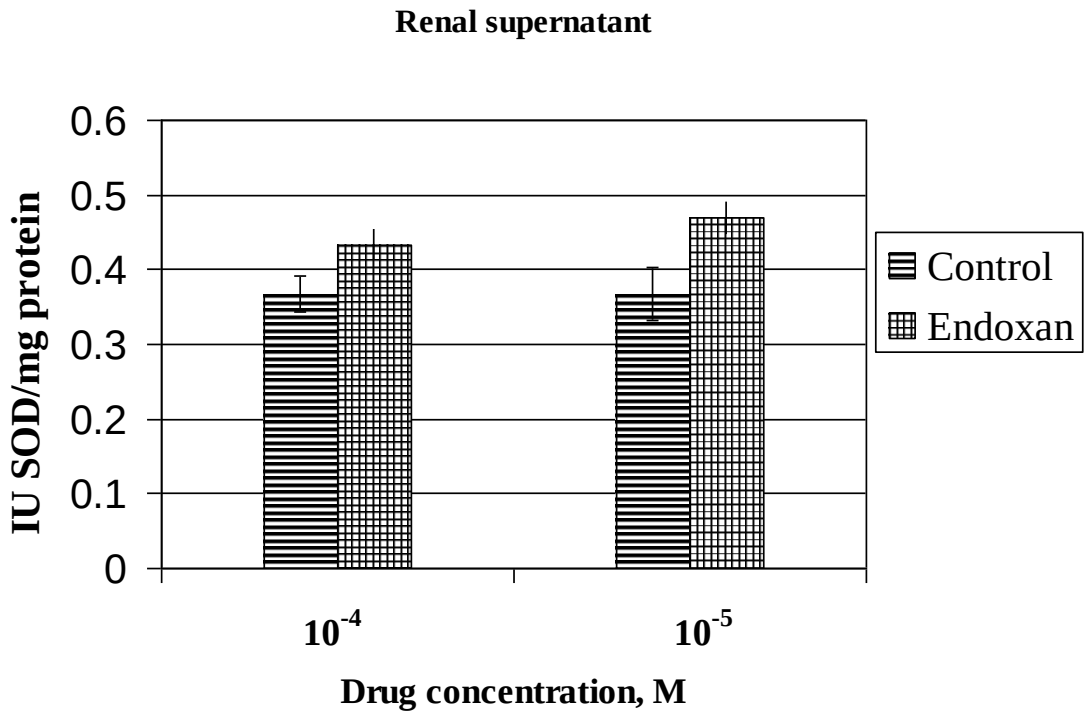


Figure 2 ***Effect of endoxan on the level of SOD concentration in renal supernatant.***

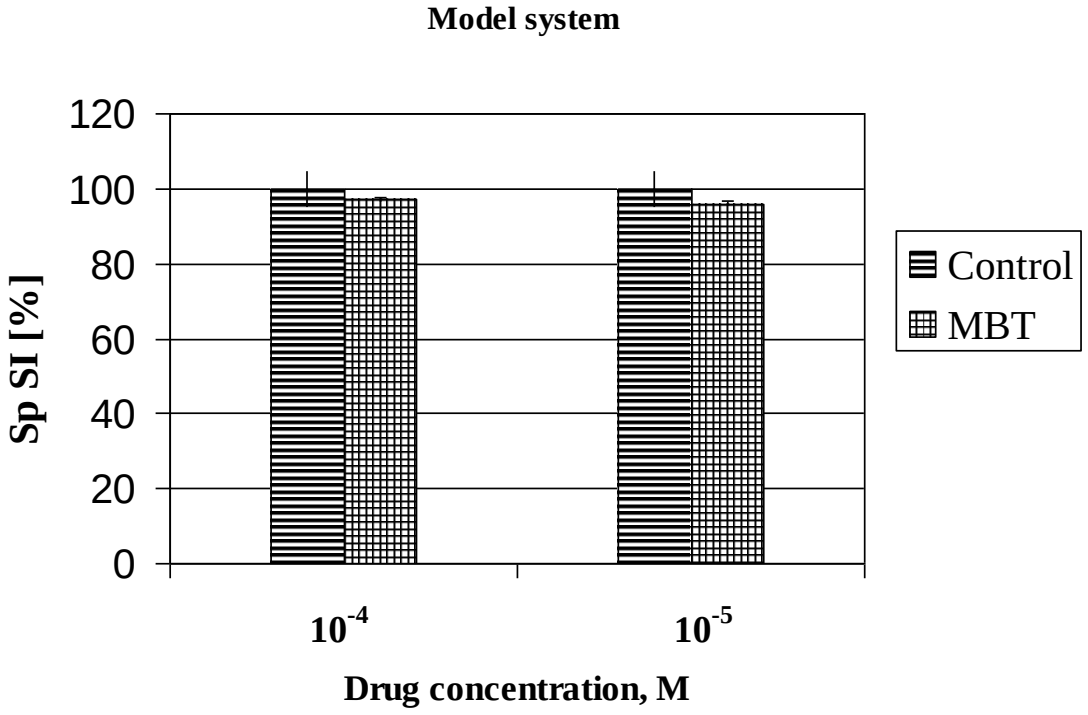


Figure 3 ***Effect of endoxan on the level of MBT in renal supernatant.***

Discussion

The results obtained with the described experiments suggest that the observed prooxidant effect of endoxan *in vivo* is not connected with its direct effect upon the Fe^{2+} - induced free radical processes in organism. Probably this effect of endoxan is connected with its metabolism from the renal monooxygenases leading to receiving of products with prooxidant effect.

Conclusions

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

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