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Effect of doxorubicin and some boron compounds on erythrocyte fragility in rats

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Abstract

Objective: Objective: Doxorubicin is a potent antineoplastic agent used in chemical treatment of cancer. However due to their cardiotoxic effects, its use is limited and currently subject for extensive research. Cardiomyopathy caused by doxorubicin still remains as a major concern for this potent drug. This study was aimed to contribute to this issue which needs novel preventive treatment for this side effect.

Materials and Methods: Boron and boron containing molecules are subject of medical research due to their properties such as antioxidant nature. They are tested in various diseases and experimental setups. In this study Boric acid, Potassium tetraborate, Amonium biborat tetrahidrat was given to male rats at 10 mg/kg/day for 6 weeks. All study groups (n=8 each) received Doxorubicin and Boron containing compounds via intraperitoneal route. Following 6 weeks blood was withdrawn and erythrocyte osmotic fragility test was applied.

Results: Erythrocyte fragility values of all doxorubicin containing groups increases and become significant at 0.5, 0.6, 0.7 and 0.8% NaCl concentrations ($p<0.05$). Among doxorubicin groups no significant difference was observed. Results of this study suggests that doxorubicin increases erythrocyte osmotic fragility and boron containing molecules are not sufficient to recover this untoward effect on erythrocytes at the applied dosage.

Conclusion: Possible mechanisms of this result and testing in a dose dependent manner can be tested in future studies.

Keywords: Doxorubicin, Cardiotoxicity, Boron, Erythrocyte Fragility, Rat

Introduction

Erythrocytes have a major role in oxygen transport. Their high surface/volume ratio enables them to pass through narrow capillaries which are smaller than their diameter (1). Although cardiovascular health focuses on blood vessels and cardiac tissue, erythrocytes may also influence cardiovascular health (2). Erythrocyte aggregation and deformability are among the important contributors of blood viscosity which increases cardiovascular diseases risks (3). Erythrocytes are prone to various stresses during their 120 days of lifetime period. They may be affected from oxidative stress or chemicals that harm to their membranes (4). Erythrocytes are subject to scientific research since impact of different chemical accumulates on them since they have no nucleus or organelles to alleviate these harmful impacts. Erythrocyte fragility is an important indicator of erythrocyte integrity. Some chemicals may increase erythrocyte fragility (5).

Most of the compounds used in cancer therapy are highly cytotoxic and many of them possess mutagenic activity.

Doxorubicin is a widely used anticancer drug used in human neoplasms. In addition it is mutagenic and carcinogenic which is known to cause chromosome damage. Reduction of doxorubicin ends up with free radicals which cause DNA fractures, lipid peroxidation, alkylation of proteins and DNA. That carcinogenic and cardiac side effect limits its clinical use although it provides successful. There are efforts to overcome doxorubicin cardiotoxicity with different chemicals such as octreotidine (6). Boron containing compounds are other molecules for this activity since they possess different activities such as preventing cancer cell proliferation (7) and extracellular cell formation (8).

Harmful effect induced by doxorubicin includes free radicals generation and triggering of antioxidant enzymes synthesis (9). This fact reminds antioxidant therapy trials. Literature records report balancing activity of Boron on tissue oxidant-antioxidant status (10). Boron may increase glutathione which is an antioxidant agent and other similar agent levels so neutralize reactive oxygen species.

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This alleviates and prevents this oxidative damage. Whoever, no literature information exists on the effect of doxorubicin on erythrocyte fragility.

This study aims to assess the effects of different boron containing compounds such as boric acid, borax, ammonium biborate tetrahidrat on the erythrocyte fragility of rats.

Material and Methods

Animals

40 male Wistar albino rats were used weighing 200-220 g. Water and food was given ad libitum to animals kept in plastic cages. Temperature and humidity was kept in constant conditions. 12 hour dark/12 hour light period was set at the animal husbandry unit.

Drugs

During experimental procedure 10 mg/kg boron (B) was used. This dose was set according to literature records. This dose cause biological impact without unwanted side effects. Doxorubicin (Dox) (Sigma) at 2.5 mg/kg once a week will be used for 6 weeks and totaling 15 mg/kg for each animal. Boric acid (BA) (Sigma), Potassium tetraborat (KTB), Amonium biborate tetrahidrat (ABB) was given at 10 mg/kg/day for 6 weeks. All study groups received Dox and B compounds via intraperitoneal route.

Groups and study protocol

Five groups were formed. Each group consists of 8 animals. Control group received standard pellet and water. Those study groups received 2.5 mg/kg of Dox once a week. This administration was continued for 6 weeks and a total of 15 mg/kg Dox was administered to all of the animals.

Third group was set as Dox and BA. Fourth group received Dox and KTB. Fifth group received Dox and ABB. Blood was withdrawn from the heart of animals into anticoagulant coated tubes under anesthesia at the end of 6th week of experimental protocol.

Erythrocyte fragility measurements

Erythrocyte fragility was assessed by the help of a spectrophotometric device (Prime-Ev, BPC). Dilutions including Na2HPO4, NaH2PO4 buffers were set as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.85 % NaCl. Blood was withdrawn into K-EDTA containing tubes. After 24 hours of incubation at room temperature 30 microliters of blood was transferred into 9 test tubes containing 2 ml of dilution solutions. Tubes were mixed with solution. After 30 minutes of incubation at ambient temperature, tubes were centrifuged for 5 minutes at 3000 rpm. Plasma fraction of the centrifuged tubes placed into the spectrophotometer. Adsorbance was measured at 546 nm.

Statistics

Results are expressed as mean ± standard deviation. For statistical analysis Kruskal-Wallis test was used. Significance was accepted as $p<0.05$. SPSS (ver: 13) software was used as the statistical program.

Results

Erythrocyte fragility

Results of erythrocyte fragility show that fragility values of all doxorubicin containing groups increases significantly at 0.5, 0.6, 0.7 and 0.8% NaCl concentrations ($p<0.05$). No significant difference was observed among doxorubicin groups (Table 1, Fig. 1).

Table 1. Results of erythrocyte fragility at different concentrations of NaCl

	0,1 % NaCl	0,2 % NaCl	0,3 % NaCl	0,4 % NaCl	0,5 % NaCl	0,6 % NaCl	0,7 % NaCl	0,8 % NaCl	0,85 % NaCl
Control	100	94	90	87	49	1	1	0	0
Dox	100	92	89	87	78*	33*	15*	3	0
Dox + BA	100	97	92	88	77*	44*	19*	9	0
Dox + KTB	100	96	95	91	86*	47*	12*	2	0
Dox + ABB	100	93	87	85	74*	52*	25*	6	0

Erythrocyte fragility results of groups: Numbers indicate percent hemolysis. n=6 rats for each group. Results were presented as percent hemolysis at different concentrations of NaCl. * $p<0.05$.

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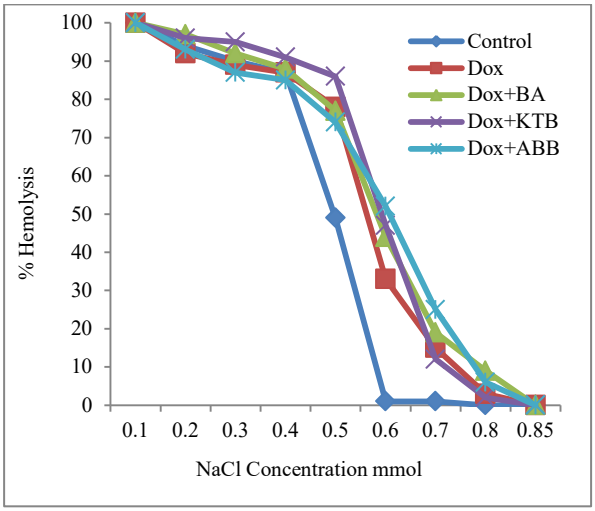

Figure 1. Erythrocyte fragility results of groups. Results were presented as percent haemolysis at different concentrations of NaCl. *p<0.05.

Discussion

This study was conducted to show any possible impact of doxorubicin on erythrocyte fragility and to assess boron containing chemicals on this activity.

Erythrocytes are major cellular elements in blood and play role in cardiovascular diseases with their aggregation and deformability properties (11). Attenuated erythrocyte deformability or augmented aggregation properties of them may end up with increased risk of cardiovascular events (12). Most of those alterations in erythrocyte functioning is linked with their membrane properties. Therefore erythrocyte membrane stability tests are providing information for their integrity and proper functioning. Results of this study present that doxorubicin increases erythrocyte fragility compared to control group. This increase is significant between 0.5 to 0.7% NaCl dilutions.

As reported in scientific literature doxorubicin is a potent stimulator of oxidative stress in organisms (13). This oxidative stress causes various side effects which limits the clinical use of this potent antineoplastic drug (14). Results of erythrocyte fragility in this study also support literature knowledge. Erythrocytes become more vulnerable to osmotic stress of increasing distilled water in dilutions following doxorubicin administration. This finding suggests a negative impact of oxidative stress on membrane stability and integrity of erythrocytes triggered by doxorubicin.

Boron containing molecules were shown to ameliorate cyclophosphamide induced oxidative stress effects in Wistar rats (15). Therefore a similar outcome in this study was also expected. Since erythrocytes are known to be affected by oxidative stress negatively any molecule with antioxidant property is expected to attenuate this detoriating effect of oxidative stress molecules. However mechanism responsible from this result obtained in fragility test might not be directly related with oxidative stress and may be due to other routes such as alterations during hematopoiesis. Boron compounds are known as protective for hematopoiesis against heavy metal toxicity (16), protective for lymphocytes at 5 and 10 mg/L (17) and known to alter blood profile (18).

Assessment of boron dosage is also needed since effects of boron may change with applied dosage. In a study by Hu et al. (19) boron at 40 mg/L was shown to exert improvement in antioxidant capacity and other biomarkers in spleen whereas at 80 mg/L it detoriates such parameters and cause organ damage. Boron derivatives are known to influence reproductive system and sperm quality in rodents and dogs at certain doses. No adverse effect dosage was determined as 17.5 mg/kg daily (20). Therefore dosage chosen in this study can be considered as safe in this aspect. However, daily consumption and limitations for human are requires further dose studies.

Conclusion

As a conclusion, our results clearly proved that ATT is non-genotoxic and it has an important antioxidant potential. Moreover, it did not change TOS levels at all concentrations. However, more in vivo studies are required before it can be used to environmental and biological applications.

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Conflict of interest: The authors declare that there are no conflicts of interest

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