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The protective effects of Selenium and Boron against Cyclophosphamide-induced bone marrow and blood toxicity: An *in vivo* study

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Abstract

Thanks to their antioxidant, anti-apoptotic, anti-lipid peroxidative, and immune-boosting properties, Boron (B) and Selenium (Se) are essential trace elements for the human body. This study aims to compare the myeloid protective potentials of Se and B in Cyclophosphamide (CP)-induced bone-marrow and haematological toxicity in experimental rats considering that the myelotoxic property of this anti-cancer drug limits its use. We hypothesized that selenium has a better protective effect than boron in preventing the toxic effects of CP on bone marrow and blood cells. 1.5 mg/kg of Se and 20 mg/kg of B, which are the most frequently used optimal doses of these trace elements, were given to the animals intraperitoneally throughout the experiment. 200 mg/kg of CP was administered only on the 4th day. The animals were sacrificed to take the blood and bone marrow samples to be stored for hematological evaluations. The CP administration significantly decreased leukocyte (WBC), thrombocyte (PLT), erythrocytes (RBC), and bone marrow nucleated cell counts. On the other hand, they increased in significant amounts in the groups given Se and B along with CP when compared to those given only CP. However, Se proved to be more protective than B in preventing CP-induced bone marrow and hematologic toxicity despite not achieving statistical significance. It was, therefore, concluded that the doses used in this experiment were successful in protecting against CP-induced damage to the bone marrow and CP-related hematological toxicity.

Key words: Cyclophosphamide, Selenium, Boron, Bone marrow, Hematological toxicity

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Selenyum ve Bor'un Siklofosfamid kaynaklı kemik iliği ve kan toksisitesine karşı koruyucu etkileri: Bir *in vivo* çalışma

Özet

Bor (B) ve Selenyum (Se), antioksidan, anti-apoptotik, anti-lipid peroksidatif ve bağışıklık güçlendirici özellikleri sayesinde insan vücudu için gerekli eser elementlerdir. Bu çalışma, Se ve B'nin Siklofosfamid (SFD) ile

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indüklenen kemik iliğinde miyeloid koruyucu potansiyellerini ve bu anti-kanser ilacın miyelotoksik özelliğinin kullanımını sınırladığını göz önünde bulundurarak deneysel sıçanlarda hematolojik toksisiteyi karşılaştırmayı amaçlamaktadır. SFD'nin kemik iliği ve kan hücreleri üzerindeki toksik etkilerini önlemede selenyumun bordan daha iyi bir koruyucu etkiye sahip olduğunu varsaydık. Bu eser elementlerin en sık kullanılan optimal dozları olan 1.5 mg/kg Se ve 20 mg/kg B hayvanlara deney boyunca intraperitoneal olarak verildi. Sadece 4. günde 200 mg/kg SFD uygulandı. Hayvanlar sakrifiye edilerek hematolojik değerlendirmeler için kan ve kemik iliği örnekleri alındı. SFD uygulaması, lökosit (WBC), trombosit (PLT), eritrositler (RBC) ve kemik iliği çekirdekli hücre sayılarını önemli ölçüde azalttı. Öte yandan, SFD ile birlikte Se ve B verilen gruplarda, sadece SFD verilenlere göre önemli miktarlarda arttı. Ancak Se'nin, istatistiksel anlamlılık elde edememesine rağmen, SFD'nin neden olduğu kemik iliği ve hematolojik toksisiteyi önlemede B'den daha koruyucu olduğu kanıtlanmıştır. Bu nedenle, bu deneyde kullanılan dozların, kemik iliğinde SFD'nin neden olduğu hasara ve SFD'e bağlı hematolojik toksisiteye karşı korumada başarılı olduğu sonucuna varıldı.

Anahtar kelimeler: Siklofosfamid, Selenyum, Bor, Kemik iliği, Hematolojik toksisite

1. Introduction

Antineoplastic medications used for treating cancer are meant to either regress the neoplastic disease or halt its progression. A great deal of research into humans and animals has shown various antineoplastic drugs that are used in treating cancer harbor carcinogenic potential [1]. Such alkylating compounds as Cyclophosphamide (CP), Carmustine, Chlorambucil, and Procarbazine are the most carcinogenic antineoplastic medicines [2]. What particularly limits the chemotherapeutic dose of sensitizing medicines is that they inhibit the immune system [3], as a result of which leukopenia and thrombocytopenia have been reported. Therefore, these drugs significantly decrease the chances for an effective therapeutic impact with higher doses and/or more frequent use [4].

CP is a strong medication that is commonly used in treating acute and chronic leukemia, breast cancer, myeloma, and bone marrow transplants [5-7]. The common side effects of this drug are hematopoietic depression, hematotoxicity, hemorrhagic cystitis, renal toxicity, and bone marrow suppression [5]. Phosphoramide mustard (PAM) and acrolein (ACR) are two active CP metabolites. PAM is linked to the antineoplastic actions of CP [5]. PAM is believed to bind to DNA and inhibit cell division, thus causing immunosuppressive and anticancer effects. The active metabolite ACR is linked to the toxic action of CP. By disrupting the tissue antioxidant (AO) defense system, ACR triggers high levels of Reactive Oxygen Species (ROS) production [8, 9]. ACR produces free radicals, which affect enzymes, receptors, and ion pumps, among other things. To avoid the toxic side-effects of ACR during a neoplastic disease and CP chemotherapy, certain AO agents are used [10].

Known for its antioxidant activity, Se is an essential trace element that functions as a cofactor of many enzyme types in the human body. It plays a role in many metabolic processes involving the glutathione peroxidases (GPx) and selenoprotein, both of which protect cells from oxidative damage. GPx is an antioxidant enzyme found in the cytoplasm that serves as a storage of Se [11, 12]. By interacting with vitamin E, Se protects cellular membranes against oxidative effects of the peroxides that come about as a result of the lipid metabolism [13, 14]. Besides protecting the function of the membrane inhibiting lipid peroxidation [15], Se is reported to exert a synergistic effect with chemotherapeutic agents thanks to its interactivity with antioxidants, thus augmenting their therapeutic effect [16], and decreasing the toxic side-effects of cisplatin. It is also reported to have protective effects upon CP-induced bone marrow and hematological toxicity [11].

Used in traditional medicine, B is a mineral that is found in the forms of boric acid and borax in nature. It is also used in industrial, agricultural, and cosmetic applications [17-20]. It is consumed in the form of boric acid, which is absorbed by the digestive system and distributed by body liquids, as part of everyday diet [21]. Studies have emphasized the renoprotective [22], antioxidant [23], hematoprotective [8], hepatoprotective [17], and antigenotoxic [20, 24], effects of this acid. Also, boric acid limits oxidative damage by augmenting the glutathione storage of the body and inhibiting other ROS [22, 25]. While there is known to be scientific research into the biological properties of various trace elements [26], those of Se and B have been investigated by a very limited number of studies. Therefore, the present study aims to compare the protective effects of Se and B upon CP-induced bone marrow and hematological toxicity in rats.

In this study, we hypothesized that selenium and boron have a protective effect in preventing the toxic effects of CP on bone marrow and blood cells due to their cytoprotective and intracellular antioxidant system strengthening effects. Additionally, we assumed that selenium would have a statistically better effect than boron.

2. Materials and methods

2.1. Materials

Boric acid with a purity of 99%, B compound, Se (Sigma-Aldrich, Darmstadt, Germany), and CP (Endoxan) were commercially purchased. B, Se, and CP were administered intraperitoneally (i. p.) and were suspended in distilled water. Their dosages were adjusted according to the research data available in the literature [17].

2.2. Experimental animals and treatment protocols

An experimental study was conducted following the official permit obtained from the Animal Experiments Local Ethics Committee of Eskişehir Osmangazi University (No: 148/776-1). All of the animals were purchased from Kobay I.C. Experimental Animal Production Laboratory of the Ministry of Health of the Republic of Turkey. The animals were kept under standard conditions (45-50% humidity, 22 ± 2° C temperature, and 12 hours light/12 hours dark) where drinking water and standard pellet bait were used to feed them. Specifying the animal diet ingredients are shown in Table 1 [27].

Table 1. Standard animal diet ingredients (mg/kg, IU/kg, µg/kg)

Diet ingredients	mg/kg	IU/kg	µg/kg
Crude Ash	61.128		
Crude Fibre	45.480		
Crude Fat	51.398		
Crude Protein	225.155		
Carbohydrates	404.451		
Calcium	7.062		
Potassium	10.144		
Magnesium	2.055		
Sodium	2.154		
Phosphorus	5.090		
Trace elements			
Aluminium	81.85		
Chlorine	3,382		
Iron	191.02		
Fluorine	3.05		
Iodine	1.53		
Cobalt	0.37		
Copper	13.89		
Manganese	77.69		
Molybdenum	1.54		
Sulfur	974.40		
Selenium	0.26		

Table 1. Continued

Zinc	84.99		
Added vitamins (fortified)			
A		26.250	
D3		1.050	
E	133		
K3	5		
B1	32		
B2	21		
B6	16		
B12			42
Nicotinic Acid	63		
Pantothenic acid	37		
Folic acid	4		
Biotin			295
Choline chloride	1050		
C	63		

Sprague-Dawley male rats of 200-250 g were selected for the experiment and were randomly divided into groups of 6 members: Control (Group I), CP (Group II), B (Group III), Se (Group IV) B+CP (Group V) and Se+CP (Group VI) (Fig.1). The rats used in the present study were given 1.5 mg/kg of Se and 20 mg/kg of B intraperitoneally (i.p.) for 6 days. A single dose of CP (200 mg/kg) was injected i.p. only on the 4th day of the experiment. All the blood and bone marrow samples were collected under anesthesia after 24 hours of the final applications.

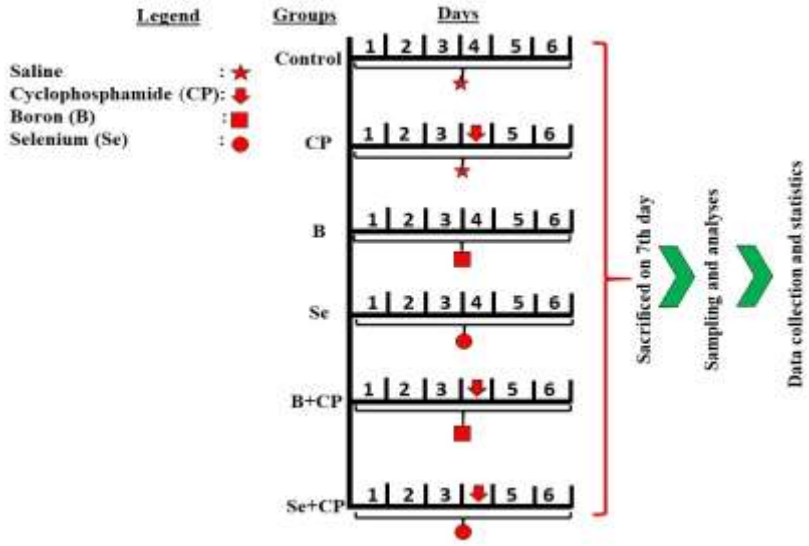

Figure 1. Experimental and treatment-related procedures of the rats

2.3. Isolation and counting of bone marrow nucleated cells

The muscles of the femur of the rats sacrificed under ketamine/xylazine anesthesia were resected [28]. Next, the femur was excised by cutting at both ends (Fig 2.1). Then, 5 mL of pressurized saline was injected through the upper end of the femur to push the whole bone marrow into a graduated test tube (Fig 2.2). This sample was then

homogenized via the same syringe that was now stripped off its needle by withdrawing and discharging the liquid content as carefully as possible a couple of times so that it would not foam up at all (Fig 2.3). The test tubes were centrifuged at 3000 rpm for 5 minutes and the supernatant was removed until 0.5 mL of the content was left behind. The pellet and supernatant (0.5 mL) were re-homogenized by pipetting the content with a Pasteur pipette. Finally, the samples obtained were counted via a blood count device [8] (Fig. 2).

Figure 2. Isolation steps of the bone-marrow nucleated cells

2.4. Measurement of Hematological Parameters

Once the cardiac blood of the animals had been harvested and poured into an EDTA tube, the hematological parameters of RBC, WBC, hemoglobin (Hb), thrombocyte, and hematocrit (Ht) levels were calculated thanks to an automated hemato-analyzer [29].

2.5. Statistical analyses

The experimental data were evaluated by using the program package version of "SPSS 18.0 for Windows". The differences between the groups were evaluated by Kruskal-Wallis One Way Analysis of Variance on Ranks. The Median value was 25-75%. If the differences observed between experimental groups were $p<0.001$, they were taken as statistically significant.

3. Results

3.1. Se and B alleviate the hematological toxicity caused by CP

Hematocrit, Hb level, RBC, WBC, and thrombocyte counts of the rats that had been given Se, and B were found to be very similar to those of the Control Group. A significant decrease was recorded in the abovementioned parameters of the rats given CP when compared to the Control Group. The same parameters also significantly improved in the rats of Se+CP and B+CP groups. However, Se seems to be slightly more effective than B in numeric expression despite being statistically insignificant (Figs.3-5).

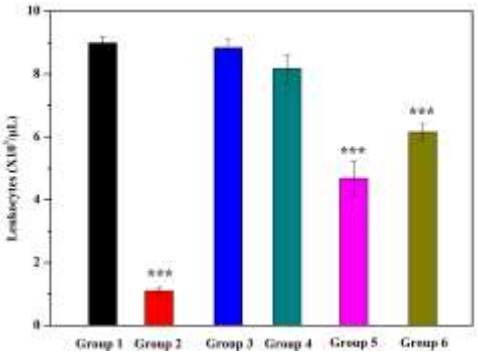

Figure 3. Comparison of the mean values of leukocyte counts of the experimental groups. *There was a difference of statistical significance between the groups marked by *** and Control Group (*** stands for p<0.001)*

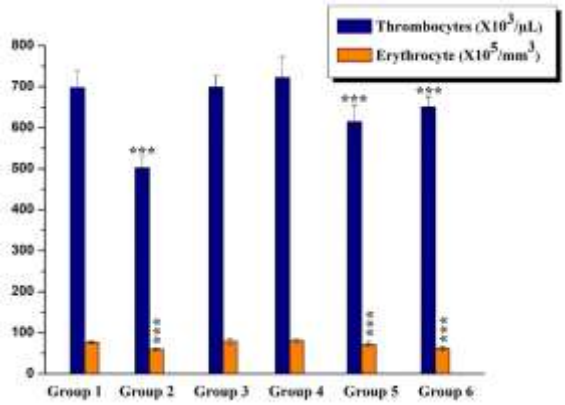

Figure 4. Comparison of the mean values of thrombocytes and erythrocyte counts of the experimental groups. *There was a difference of statistical significance between the groups marked by *** and Control Group (*** stands for p<0.001)*

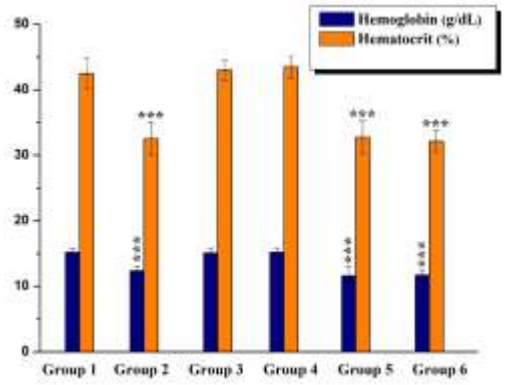

Figure 5. Comparison of the mean values of hemoglobin and hematocrit levels of the experimental groups. *There was a difference of statistical significance between the groups marked by *** and Control Group (*** stands for p<0.001)*

3.2. Se exerted a better effect on the bone marrow cellularity than did B

The bone marrow cellularity was found to be similar to that of the Control Group in the rats given Se and B. While the cellularity significantly decreased in Group 2 ($p<0.001$) when compared to the Control Group, it increased in Groups 5 and 6 when compared to Group 2. As for Se, it proved to be slightly more effective than B when expressed in numerical terms (Fig.6).

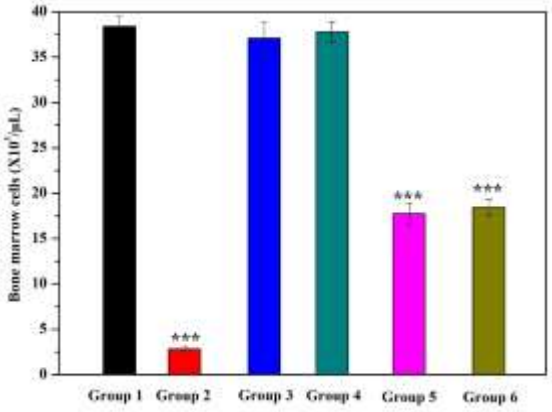

Figure 6. Comparison of the mean values of bone-marrow nucleated cell counts of the experimental groups. *There was a difference of statistical significance between the groups marked by *** and Control Group (*** stands for p<0.001)*

4. Conclusions and discussion

This study demonstrates that selenium and boron have a protective effect in preventing the toxic effects of CP. The protective effect hypothesis was confirmed by preventing significant reductions in bone marrow, WBC, RBC, PLT, Hb, and Ht levels in CP-administered rats. However, the second hypothesis, that selenium provides better protection than boron, was verified numerically, not statistically.

The number of cancer patients is on the increase worldwide, which results in the production of more and more anticancer drugs [2], with their clinical results being limited due to their adverse side effects [30]. Therefore, different treatment approaches are needed that will not only protect the anticancer potential of these drugs but also diminish their side effects as well. Recent studies have shifted their focus to using trace elements with antioxidative, anti-inflammatory, and antiapoptotic properties. CP changes the hematopoietic system as one of the most widely-used anticancer drugs with a strong myelotoxic adverse effect [31].

The present study aims to compare the protective effects of Se and B upon CP-induced bone marrow and hematological toxicity in rats. WBC, RBC, PLT, Ht, Hb, and bone marrow nucleated cell counts significantly decreased in Group 2 when compared to Control Group (Fig. 3). Our study results concerning the bone marrow and peripheral blood samples were found to be similar to those in the literature [8, 32, 33]. For example, it has been reported that 200 mg/kg CP significantly decreased the leukocyte, thrombocyte, and bone marrow nucleated cell counts compared to those of the Control Group [8]. Likewise, another study reported that CP gave in doses of 50, 100, and 150 mg/kg decreased the counts of leukocytes, thrombocytes, and bone marrow cell counts in rats [32]. Still another study reported that 200 mg/kg CP administration resulted in a drop in WBC, thrombocytes, and bone marrow cells [33].

Se is a trace element known as a component of selenoproteins (GPx and thioredoxin reductase) that modulate the redox state of the cells necessary for the endogen antioxidant enzyme balance. Selenoproteins do protect the cell against the effects of oxidative stress. Due to their abovementioned properties, they are capable of protecting cells against the adverse effects of anti-cancer drugs like CP [32, 34]. In the present study, RBC, WBC, Hb, thrombocyte, and bone marrow cell counts significantly decreased in the rats given a single dose of CP, however, these decreased values significantly improved when Se was added to CP (Figs.3-6). These results are compatible with those published in the literature [32, 34].

While antioxidant mechanisms of B still remain to be clarified, it plays a crucial role in cellular membrane activities [35]. Previous studies have reported B to strengthen the antioxidant defense system, decrease lipid peroxidation, and significantly alleviate the bone marrow and hematological toxicity caused by CP. In the present study, a single dose of CP resulted in a decrease in the parameters of WBC, Hb, RBC, platelet, bone marrow, and hemoglobin levels. However, a significant improvement was noted in the values indicated following the administration of 20 mg/kg B together with CP (Figs.3-6). Our study results are consistent with those available in the literature [8].

CP is assumed to inhibit the production of erythrocytes by suppressing the bone marrow, as a result of which haematocrit values get reduced. The present study showed that not only Se but also B had increased bone marrow nucleated cells erythrocytes in number, meaning that these two trace elements appear to play a protective role on stem cells. That is to say, our study results suggest that a separate or combined use of Se and B may provide a supportive approach to reducing chemotherapy complications attributable to CP. Therefore, Se and B could serve as potential candidates for cell nutrition and storage in stem cell studies.

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Author Declarations

The authors of this publication have no conflicts of interest among themselves or with the institutions.

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