Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Hematoprotective effect of boron on cyclophosphamide toxicity in rats

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Received February 7, 2018; **Accepted** April 15, 2018; **Published** April 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.5.10

Abstract: The goal of this study was to determine the effects of boric acid (B) as a boron source on blood cells and indirectly on bone marrow. Intraperitoneally (i.p.) administration of 200 mg / kg of cyclophosphamide (CP) resulted in reductions in the number of erythrocyte (20%), hemoglobin (20%), leukocytes (96%), thrombocytes (41%), and hematocrit (21%). The group given CP alone was killed 3 days after the CP administrated. For the group having CP+B (200 mg/kg i.p) treatment was started 3 days earlier than the CP administration and continued to the finish of the experiment (6 days). On day 4, the animals were weighed again, relative doses of CP were expected, and CP+B was administered together. On day 7, blood samples were collected under anesthesia. The results suggest that B could reduce CP -induced toxicity on blood cells and bone marrow in rats.

Key words: Cyclophosphamide; Boron; Hematotoxicity; Cytoprotectivity; Rats.

Introduction

In cancer treatment, antineoplastic drugs are used to regress or stop the process of neoplastic disease. It has been proven in many studies on people and experimental animals that some of the antineoplastic drugs used in cancer treatment have carcinogenic potential (1,2). The most carcinogenic of antineoplastic drugs are the alkylating agents such as CP, carmustine, chlorambucil, procarbazine (3). The mainly important effect that restricts the chemotherapeutic dose of the sensitizing drugs is that they are immunosuppressive (4). Depending on the immunosuppressive effects of the sensitizing drugs, leukopenia, thrombocytopenia and lymphopenia develop. This prevents them from achieving a stronger therapeutic effect with higher doses and / or more frequent use (5). Cyclophosphamide is a powerful drug widely used in acute and chronic leukemia, breast cancer, myeloma bone marrow transplants (6-7). The major side effects of CP are hematopoietic depression, hemorrhagic cystitis and renaltosis. In addition, it has thoughtful toxic effects such as hematoxicity, urotoxicity, teratogenicity, mutagenicity, carcinogenicity and suppression of bone marrow (6). Two active metabolites of CP are phosphoramid mustard (FAM) and acrolein (ACR). The antineoplastic effects of CP are associated with FAM. It is believed that FAM binds to DNA and suppresses cell division, thus mediating immunosuppressive and antitumor effects. The toxic effect of CP is related to ACR, which is the active metabolite. ACR interferes with the tissue antioxidant (AO) defense system, leading to high SOR formation (8). Free radicals formed by ACR; enzymes, receptors, ion pumps, and so on. During neoplastic disease, during CP chemotherapy, these toxic effects should be detoxified by using some AO agents to avoid the intoxic side effects of ACR (7). Although methods have been developed that allow the use of higher doses by avoiding the toxic effects of CP, currently drug administration systems are in search of methods that may be more sensitive. B is a naturally occurring mineral that is widely used in health, industry, agriculture and cosmetics (9). In many studies, it was emphasized that B has antioxidant, hepatoprotective and antigenotoxic effects (10). It has also been suggested that B promotes glutathione in the body and inhibits oxidative damage by inhibiting other reactive oxygen species (9). In the literature, no studies have been found on the possible protective effect of B on CP-induced blood cells and bone marrow toxicity. Based on these facts, the possible protective effect of B on CP-induced toxicity of the blood and indirectly bone marrow of rats were investigated in this study.

Materials and Methods

99% pure boric acid as a boron source and CP (Sigma-Aldrich, Darmstadt, Germany) were purchased from the commercial company. It was given as i.p. chemical injections. Only the animals in the 200 mg/kg CP group were anesthetized 3 days after the CP injection. Group given B+CP, 200 mg/kg B (10) were started 3 days before CP application and continued throughout the experiment. On day 4 the animals were weighed again and CP was dosed so that CP + B was given on day 4. On day 7, animals were anesthetized and blood was removed (Table 1) (11).

Experimental Protocol

Rats were randomly divided into the following experimental groups, each including six animals (table 1).

Table 1. Description of experimental groups and doses of the chemicals applied.

Groups/ Days	1.	2.	3.	4.	5.	6.	7.
Control	SF	SF	SF	SF	SF	SF	Sacrified
CP	SF	SF	SF	CP	SF	SF	Sacrified
B	B	B	B	B	B	B	Sacrified
B+CP	B	B	B	B+CP	B	B	Sacrified

SF: saline, CP: cylophosphamide; B, boric acid as a boron source.

Statistical analysis

The results were expressed as the mean ± standard error of the mean. Statistical analysis was performed followed by one-way analysis of variance (ANOVA) With Tukey's multiple interval test, $p< 0.001$ was accepted statistically. Triplicate samples taken from each animal were taken and the results were expressed as an average for each animal.

Results

Statistical comparison of control and experimental groups to mean values of erythrocyte, leukocyte, platelet, hemoglobin and hematocrit were shown in the figures and Table 2. In the 200 mg/kg B group the increase in number of erythrocyte, leukocyte, hemoglobin and hematocrit compared to the control group, was not statistically significant ($p > 0.05$), but the increase in the number of the thrombocyte (13%) (Figure 1). In the experimental group given 200 mg / kg CP, the number of erythrocytes and hemoglobin decreased by 20% and the number of hematocrit decreased by 21% compared to control group. In the experimental group in which CP + B was applied, it was determined that erythrocyte, hemoglobin and hematocrit values were close to the control group values (Table 2). When used alone, 200 mg/kg of CP caused 96% reduction in the number of leukocytes, ($p < 0.001$) compared to control group, which means that bone marrow was being seriously repress.

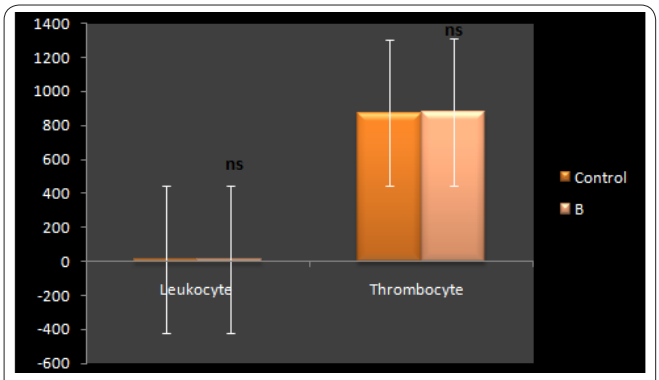

Figure 1. The number of peripheral leukocytes and thrombocytes with the presence of saline, and 200 mg/kg of B. ns; not significant compared to control group.

Table 2. Comparison of control and experimental groups to mean values of erythrocyte, hemoglobin and hematocrit number

Groups	Erythrocyte (X10⁶/mm³)	Hemoglobin g/dl	Hematocrit %
Control	7.42	14.8	41.8
CP	5.94	11.6	32.5
B	7.93	15.08	43.28
B+CP	6.27	10.36	31.4

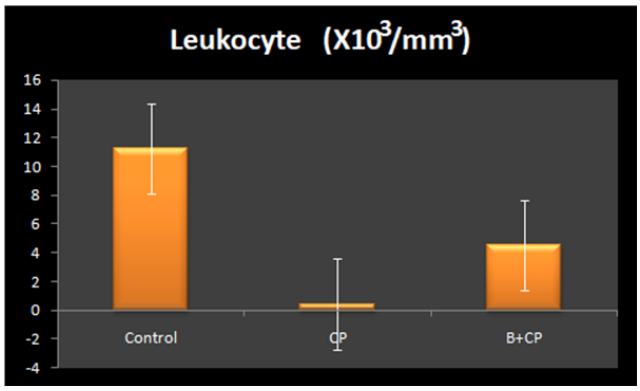

Figure 2. The number of peripheral leukocytes with the presence of saline, respective doses of CP, or CP plus B. ***; p<0,001 significant different compared to control group, **; p<0,01 different compared to control group.

Administered together with respective doses of CP+B reduced the number of leukocytes by 50% compared to control group which suggests that B improves the suppression of this affected bone marrow significantly ($p < 0.001$) (Figure 2). The number of platelets in rats treated only with 200 mg/kg of CP alone decreased by 41% ($p < 0.001$) compared to control. Administered together with only dose of CP, 200 mg/kg of B reduced the number of thrombocytes by 20% compared to control group ($p < 0.001$) (Figure 3).

Discussion

The chemotherapeutic usefulness of alkylating agents are derived from their capability to form a diversity of DNA adducts that adequately change DNA structure or function or both in an attempt to have a cytotoxic effect on the cells. Many of them go through a very complex activation process before they can generate reactive intermediates. Initial activation reaction of CP carried out by the microsomal oxidation system in the liver produces 4-hydroxy CP, a cytotoxic metabolite, which diffuses from hepatocytes into plasma and is distributed throughout the body. Then, 4-hydroxy CP is further converted to other cytotoxic metabolites, such as ACR and PAM, known to reason myelosuppression (11, 12) Myelosuppression is a major potential toxic and dose-limiting side effect of CP. CP causes cross-linking of DNA and inhibition of DNA synthesis by acting on both cyclic and inter-mitotic cells, resulting in general

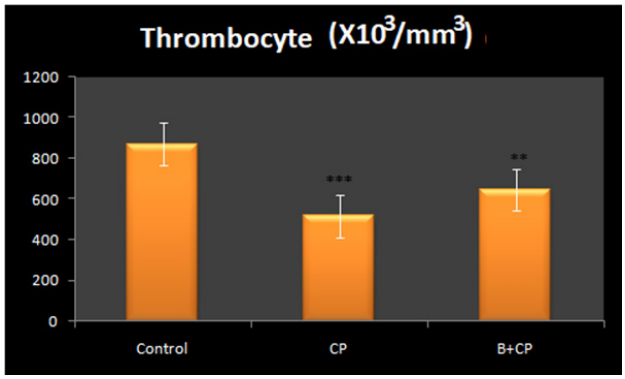

Figure 3. Blood thrombocyte number of the rats after treatment with saline, respective dose of CP, or CP plus B. ***; p<0,001 significant different compared to control group, **; p<0,01 different compared to control group.

depletion of immune component cells (13). In one study, It was reported that 150 mg / kg CP caused a decrease of 92% in the leukocyte count, 54% in the platelet count and 94% in the bone marrow (14). Similarly, after the single dose of CP injection, leukocyte counts began to fall (15). Further, administration of a single dose of 40 mg/kg CP to baboons resulted in transient reduction in WBC count (16). Another experimental study has been emphasized that CP (20 and 40 mg/kg) has a mutagenic effect on spleen and bone marrow (17). Trasler et al. (18) emphasized that the effects of erythrocyte and leucocyte counts on bone marrow cell count and bone marrow in CP mice treated with erythrocyte and leucocyte counts were very dramatic if high dose CP was given. The results of this study are similar to the studies mentioned above.

B plays a role in cellular membrane functions, even though the antioxidant mechanisms of its have not yet been clearly defined (19). In the previous study, it was emphasized that 200 mg / kg of B decreased the lipid peroxidation and increased the antioxidant defense system (10). B is an essential element for metabolic processes in living organisms (19, 20). Boric acid contains 17.48% boron. Boron does not accumulate in human body (21). The current data show that oral LD50 values for boric acid in mice and rats are in the range of about 400-700 mg boron per kilogram of body weight (22). In rats, no toxic effect of B (200 mg / kg) was observed in the short time (7 days) (10). In this study, in the experimental group given 200 mg / kg CP, there was a decrease of 41% in platelet count, 96% in leukocyte count. However, the test group given 200 mg / kg CP + 200 mg / kg B decreased the leukocyte count to 50% and the platelet count to 20% when compared to the control group. To interpret these results, the application of B is thought to allow the bone marrow suppression caused by CP to be removed in a serious way. In conclusion, B alone is not toxic to bone marrow or blood cells, but CP is toxic to bone marrow, leukocytes, and platelets. B protects the animals from the toxic effect of CP. This information will enter our work for the first time in the literature. This results suggest that at convenient concentration of B could be a potentially effective drug in the treatment of CP-induced damage and could provide us with the hope in prevention and treatment of CP toxicity. I believe that additional experimentation should be performed to at least initially explore the underlying mechanism of B protection against CP toxicity.

Acknowledgments
This study was not financially upported by grants (The ethics commitee number: 648-1 / 2018).

Interest conflict
None.

Author's contribution
Mustafa Cengiz participated in the study design, data interpretation, and writing of the manuscript. In addition He, performed the animal experiments, refined the experimental protocols.

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