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Investigation of the Effects of Boron on Leucocyte Functions and Formed Free Radical Status in Endotoxin-Mediated Inflammation

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Research Article

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Abstract

Endotoxins are lipopolysaccharides which make up some part of the cell walls of gram negative bacteria. Endotoxemia is one of the most important mechanisms in the generation of inflammation. Inflammation is a powerful physiological response to tissue damage caused by the microorganisms, physical, chemical and other factors. Many cells, especially leukocytes, and the cytokines released from these cells play role at the start of inflammation. The most significant players in inflammation are interleukins (IL) and tumor necrosis factor alpha (TNF-α). The pathogens which cause inflammation are removed through phagocytosis. The oxygen-dependent mechanisms that phagocytic cells use to remove damaging factor include superoxide radical formation and the myeloperoxidase (MPO) system. Boron is an element which has a considerable importance in terms of reserve in our country. Since its areas of use are quite wide the exposure to boron is more in all respects. The boron is thought to have numerous effects on the living organisms. In the light of this information, this study aims to investigate the effect of boron on the functions of leukocyte and the situation of formed free radical in the endotoxin-mediated inflammation. Accordingly, 3 separate experimental groups each of which has 6 rats were created; control, boron+ endotoxin and endotoxin groups. 100 mg / kg boric acid was administered to boron + endotoxin group through gavage for 28 days. On day 28, 4 mg / kg lipopolysaccharide (endotoxin / LPS) were administered to endotoxin group and boron + endotoxin group intraperitoneally. In serums of rats, TNF-α and IL-6 were measured with ELISA as SOD and MPO were measured spectrophotometrically. TNF-α concentration decreased in the boron + endotoxin and endotoxin groups compared to the control group whereas IL-6 concentration did not change between the groups. SOD concentration increased in the Boron + endotoxin group compared to the endotoxin group. SOD value measured in the endotoxin group is lower than the control group. MPO concentration of the boron + endotoxin group is higher than those of the endotoxin and control groups. In conclusion, it is thought that boron has no effect on TNF-α and IL-6 levels. Increase in SOD level due to boron can be attributed to the role of boron as a regulator by affecting the production of free radicals positively. The increase in MPO level suggests that the boron element plays a useful and adaptive role in the destruction of the infectious agent taken by phagocytosis.

Keywords: Endotoxin; Leukocyte; Free radical; Boron

Abbreviations: IL: Interleukins; TNF-α: Tumor Necrosis Factor Alpha; MPO: Myeloperoxidase; LPS: Lipopolysaccharide; SOD: Superoxide Dismutase; GUDAM: Gazi University Laboratory Animal Breeding and Experimental Research Center

Introduction

Endotoxins are lipopolysaccharides (LPS) found in the cell wall structure of gram-negative bacteria. They are not released out of the cell. Instead, they liberate when they are exposed to lysis. Endotoxemia is one of the most important mechanisms for the generation of inflammation. Inflammation is a powerful physiological response to tissue damage caused by the microorganisms, physical, chemical and other factors. In other words, it is a protective response to the destruction of harmful stimuli such as microbial infection or tissue damage.

Tissue macrophages, dendritic cells and mast cells recognize the infection firstly. The production of the mediators such as chemokines, cytokines, vasoactive amines, eicosanoids and proteolytic chain products secreted by these cells increase gradually. These mediators have role in the formation of regional inflammatory exudate by attracting the leukocytes. There are numerous common features of interleukins (IL) and TNF-α which are released after excitation of leukocytes. TNF-α is responsible for eliminating the pathogens and killing the tumor cells. [1,2]. Besides, IL-6 is a molecule of the cytokine cascade that regulates the immune inflammatory response, a crucial component of the body defense [3,4].

Phagocytosis removes the pathogens which lead to inflammation. Phagocytic cells have oxygen-dependent and oxygen-independent mechanisms to eliminate the damaging factor. The substances in the granules of leukocytes draw the attention in the oxygen-independent killing mechanisms. Oxygen-dependent mechanisms contain the myeloperoxidase (MPO) system and another system that enables the production of oxygen-derived free radicals. The pathogen that spreads through the body is recognized by the members of immune system and phagocytized. NADPH oxidase system in the cell membrane of leukocyte converts the oxygen to superoxide. Rapid consumption of oxygen with the formation of superoxide is called respiratory burst. Superoxide is spontaneously converted to hydrogen peroxide. Hydrogen peroxide is converted to hypochlorous acid with MPO and chloride ions, thus this substance causes the death of the pathogen in phagolysosome. Excess superoxide in the phagolysosome or the superoxide which avoids from phagolysosome is converted to hydrogen peroxide through superoxide dismutase (SOD) activity. The resulting hydrogen peroxide is neutralized by catalase and glutathione peroxidase enzymes [5,6].

Boron is an element which has a considerable importance in terms of reserve in our country. The living creatures are exposed to boron in the air, water and soil in which they exist. Since the areas of use are quite wide, the exposure to boron is more in all respects [7-9]. The findings obtained from human experiments showed that boron is an effective dynamic trace element which is influential on the metabolism and the regulation of the effective substances for living creatures such as calcium, copper, magnesium, nitrogen, glucose, triglycerides, active oxygen and estrogen and in metabolism. Besides these effects, the structural and functional effects of boron on the body systems such as blood, brain and skeleton make it a physiologically beneficial element even though it is not considered as an essential mineral [10].

In the light of this information, the inflammation was enabled by administering LPS to experimental animals intraperitoneally. The objective was to analyze the effect of boron element on the leukocyte functions and the status of free radical by detecting TNF-α, IL-6, myeloperoxidase (MPO) and superoxide dismutase (SOD) levels.

Material and Methods

Study group

A total of 18 male Wistar Albino rats each of which weigh 350g on average and are provided by the Gazi University Laboratory Animal Breeding and Experimental Research Center (GUDAM) were used in the experiments. In each group, 3 separate experimental groups, each having 6 rats were created; control, boron + endotoxin and endotoxin groups.

In boron + endotoxin group, 100 mg boric acid per kilogram of body weight was administered to each rat without cooling through gavage for 28 days by adding to distilled water at 50 ° C and applying vortex. Considering that the stress generated during gavage may cause some biochemical parameters to change, only distilled water was given to the animals of the control group and endotoxin groups through gavage for 28 days. 4 mg/kg lipopolysaccharide (endotoxin/LPS) was administered to boron + endotoxin and endotoxin groups intraperitoneally (IP) at the end of day 28.

All groups were sacrificed 6 hours after LPS administration under the anesthesia ketamine (60 mg/kg) and xylazine (10 mg/kg). SOD and MPO concentrations in the serum samples were measured

spectrophotometrically whereas TNF-α and IL-6 were measured via ELISA.

Determination of TNF-α in serum

TNF-α concentration in serum was analyzed in ELISA using a commercial kit.

Designation of IL-6 in serum

IL-6 concentration in serum was analyzed in ELISA using a commercial kit.

Determination SOD in serum

SOD concentration in serum was analyzed spectrophotometrically [11]. The sample was centrifuged at 7000 RPM at + 4°C for 30 min. Supernatant was taken. 1 mL of ethanol-chloroform mixture was added to 1 mL supernatant. 2.45 mL reagent mixture, 0.5 mL supernatant and 50 mL xanthine oxidase were added for the sample. 2.45 mL reagent mixture, 0.5 mL distilled water and 50 mL xanthine oxidase was added for the blind. All tubes were exposed to incubation at 25°C for 20 min. After the incubation, the reaction was stopped by adding 1 mL CuCl2 to all tubes. All tubes were read at 500nm by a spectrophotometer.

Determination MPO in serum

MPO concentrations in serum were measured spectrophotometrically. The sample was centrifuged at 10000 g for 5 min at +4°C. Supernatants were taken after the centrifuge. 1000 µL phosphate buffer, 100 µL H_2O_2, 200 µL o-dianisidine, 40 µL supernatant, and 360 µL distilled water were added for the sample while 1000 µL phosphate buffer, 100 µL H_2O_2 and 200 µL o-dianisidine were added for the blind. All tubes were exposed to incubation at 37°C for 30 min. After the incubation, 200 µL 3 M HCl was included in the samples and the blind. All tubes were vortexed and mixed and read at 450 nm by a spectrophotomer.

Statistical Evaluation

Mann-Whitney U test was used to evaluate the findings. All values were given as arithmetic mean ± standard deviation and $p < 0.05$ was considered as statistically significant.

Results

The results of TNF-α, IL-6, SOD and MPO levels obtained from the experimental animals are given in Table 1.

(n=6)	TNF-α (pg/ml)	IL-6 (pg/ml)	SOD (µmol/ml)	MPO (U/min/ml)
Control	65,33±20,75	212,92±45,59	38,18±1,00	162,32±38,49
Boron+Endotoxin	38,25±11,79[a]	187,08±33,99	51,93±6,97[b]	266,67±38,49[ab]
Endotoxin	46,00±3,10[a]	242,75±60,24	24,39±4,59[a]	130,58±28,16

Table 1: TNF-α, IL-6, SOD and MPO levels.
[a]p<0,05 when compared with value of control group.
[b]p<0,05 when compared with value of endotoxin group.

Conclusion

According to the data obtained in the study, a decrease in TNF-α concentration in the endotoxin and boron + endotoxin groups compared to the control group were detected. This is a statistically significant decrease. TNF-α concentration in boron + endotoxin group was found to be statistically insignificant although it decreased compared to endotoxin group. It can be considered that low TNF-α concentrations in the experimental groups administered LPS may be attributed to adaptive behavior of the body for self-protection. Existing TNF-α may show local inflammatory effect and may be used to call the mononuclear phagocytic cell elements to the region, so its amount can be reduced through TNF-α binding proteins in the defense mechanism. The absence of a significant difference in TNF- α levels between the boron + endotoxin group and the endotoxin group causes an explanation that the boron element has no effect on TNF- α concentrations in early acute phase local inflammation. When the results of the study on pigs are examined, it appears that boron supplement does not affect TNF-α concentration significantly due to high statistical deviations. This is consistent with the findings of our study [12].

The results of our study showed that there was no difference in IL-6 levels, another indication of inflammation between the groups administered LPS and the control group. One role of TNF-α in systemic inflammation is to enable IL-6 to play a role in the inflammatory process by releasing it depending on the increase in its own amount [13]. No change in IL-6 levels may be associated with that TNF-α remains at local inflammation levels and cannot release IL-6.There was no significant difference in IL-6 levels between the boron+endotoxin, endotoxin and control groups. This

result may indicate that boron does not affect IL-6 level. In literature, a study on humans reported that boron supplement has no effect on IL-6 level in parallel with our results [14].

According to the experimental data, SOD level in the endotoxin group decreased compared to the control group. This decrease indicates the use of SOD enzyme to eliminate the superoxide radical. In the boron + endotoxin group, SOD concentration was higher than that of the endotoxin group. Considering the intense interest of the boron element to oxygen, it is thought that oxygen is carried to the environment, superoxide radical forms from the carried oxygen and the amount of SOD enzyme is increased for their elimination. In a study of Hunt, et al., Hunt argued that the boron element plays a regulator role essentially in the respiratory burst [15-18]. Our hypothesis overlaps with the study of Hunt et.al. However, it is still a question of debate that on which mechanism boron performs this and that boron perform this by reducing the derivatives of reactive oxygen or raising the antioxidant capacity. Thus, further studies are required to explain it.

The amount of increased MPO in the boron + endotoxin group compared to the control and endotoxin groups is related to high SOD value in boron + endotoxin group as direct proportion. It can be argued that MPO amount is raised for the conversion of excess amount of hydrogen peroxide produced with the activity of SOD enzyme, to hypochloric acid, a bactericidal agent. Thus, it is thought that the boron element plays a role in killing the pathogen which was taken through phagocytosis.

In consequence, adding boric acid used as boron source, to diet does not alter TNF-α and IL-6 levels in LPS-induced inflammation. It makes us think that it has no effect. On the other hand, an increase in the amount of SOD used as a marker in the production of free radicals and boron use and an increase in the amount of MPO which has an important role in intracellular killing were determined. It is thought that boron elements has regulator role in the inflammatory process. The data obtained in our study shows that adding boron to diet is a question of debate, so it will be better to conduct more advanced studies [19].

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