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Evaluation of the Effect of Boron on Histopathological Changes of Atherosclerotic Plaque in Aortic Arch and Lipid Profiles in Hyperlipidemic New Zealand Male Rabbits

Robabeh Asadi[1], Javad Raouf Sarshoori[1*], Maryam Ghorbani [2,3], Mahmood Mofid[1]

1. *Dept. of Anatomy, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran*
2. *Dept. of Pharmacology and Toxicology, Faculty of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran*
3. *Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

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Corresponding Information:
Javad Raoufsarshoori,
Dept. of Anatomy, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

E-Mail: raoufsar@gmail.com

ABSTRACT

Background & Objective: Cardiovascular diseases are the most important causes of death worldwide. Atherosclerosis, as a common form of cardiovascular disease, tends to involve specific areas of the circulatory system. Boron has anti-inflammatory and antioxidant properties with potential beneficial effects. In this study, we investigated the effect of Boron on histopathological changes of atherosclerotic plaque and lipid profile in hyperlipidemic rabbits.

Materials & Methods: Male rabbits in five groups of control, sham, hyperlipidemia, treatment 1 and treatment 2 were fed on high fat diet (1% cholesterol). Treatment groups received Boron, 4 mg / kg, on the first and 20th days of experiment. Animals' weights were measured on days 1, 21 and 60. Plasma levels of Cholesterol, LDL, HDL and TG were measured by photometric method. After 60 days, Sudan IV staining method was used for macroscopic study. Hematoxylin-eosin and Masson's trichrome staining method were performed for quantitative analysis.

Results: Animals in the control and sham groups showed no significant change in serum lipid profile with no atherosclerotic plaque in aortic vessels. In the hyperlipidemia group, significant alterations in lipid profile and presence of atheroma plaques were detected. In animals receiving Boron as a protective agent, atheroma plaques were significantly less ($p < 0.05$). This was confirmed by quantitative analysis.

Conclusion: Boron ameliorates the development and progression of atherosclerotic plaques. Boron can be used alone or in combination with other drugs as anti-atherosclerotic treatment.

Keywords: Atherosclerosis, Boron, Hyperlipidemia, Atheroma plaques, Lipid profile

Introduction

Atherosclerosis is a cardiovascular problem wherein plaque forms inside arteries leading to serious complications including heart attack, stroke, or even death. Based on affected arteries including the heart, brain, pelvis, arms, limbs, and kidneys, various conditions may develop (1). Development and progression of atherosclerotic coronary arteries result in coronary heart disease. Depending on degree of artery damage, angina or heart attack occurs (2). Atherosclerosis is a silent disease which may remain undiagnosed until its life-threatening adverse effects appear. The initial mechanism is unknown; however, it seems that some risk factors promote the disorder. Age, smoking, physical inactivity, high cholesterol blood level, diabetes, high blood pressure, unhealthy diet and family history of heart disease are considered as principal risk factors (1). Numerous steps are described in atherosclerosis pathogenesis. At first, fatty streaks, and yellow discoloration of inner surface of arteries, are formed, leading to endothelial dysfunction. Lipid peroxidation and oxidized lipids in the vessel sub-intima layer, as pro-inflammatory mediators, lead to leukocyte recruitment and foam cell formation (3, 4). Chemoattractant molecules, MCP-1 and IL-8, cause direct leukocyte, monocytes and T lymphocytes migration into the vessel intima (5). Following activation of pathologic cascade, macrophages turn into foam cells, produce further cytokines and accelerate the process of atherosclerotic plaque formation (6). Plaque development can significantly restrain the vessel lumen and impair tissue perfusion which causes ischemia and subsequent serious problems.

One of the principal medications include statin drugs,which reduce cholesterol and LDL serum levels and prevent foam cell formation (7). Since inflammation and lipoprotein oxidation have key roles in atherosclerosis development, remedies with antioxidant properties have been considered (8). Besides herbal medicines, Boron, a water soluble element, is critical for cells to support metabolic events. Boron reacts with hydroxyl group in glycolipids, glycoproteins and phosphoinositides. Thus, it affects membrane integrity and calcium metabolism (9). When Boron is taken orally, it is absorbed via the blood stream rapidly and thoroughly. Some studies have reported beneficial properties for Boron such as antioxidant activity, anti-inflammation and regulation of various hormones and mediators such as calcium, magnesium, potassium, vitamin D, aldehyde dehydrogenase, xanthine oxidase, cytochrome b reductase, insulin, estrogen, testosterone, T3, T4, triglycerides and glucose (10, 11). Boron regulates inflammatory reactions in osteoarthritis states, inhibiting the inflammatory response and reducing blood level of C-reactive protein, as well as improving antioxidant defense mechanism (12).Recently, a direct correlation has been reported between higher plasma Boron concentrations and lower BMI and a more favorable cardio-metabolic risk profile (13). As Boron is involved in the regulation of signaling pathways of inflammation, oxidative stress or lipid metabolism, it can be considered as an option for preservation of a healthy cardiovascular system (14).

In this study, we hypothesized that Boron may be a potential agent to protect or treat induced atherosclerosis lesions in rabbits.

Materials and Methods

Thirty rabbits were kept individually in stainless steel cages with free access to food and water (12/12h light/dark cycle, 20± 2°C) for two months. This animal study was in accordance with ethical committee issues, number IR.BMSU.REC1397.50. Animals were arranged in five groups: the control group (with normal diet), the sham group (with corn oil diet), the hyperlipidemia group (1% chol), and the treatment groups t1 and t2. Group t1 received both chol 1% and Boron 4mg/kg from the first day and t2 received chol 1% and Boron starting on the 20th day to the end of experiment. On days 1, 21 and 60 animals were weighed and sampled.

Blood Samplings and Biochemical Values

Blood sampling to assess lipid profile, was carried out on days 1, 21 and 60. Animals were fasted 12 hours before blood sampling. After mild sedation (ketamine hydrochloride 35 mg/kg, xylazine 5 mg/kg, IM), blood samples were taken from the auricular artery of animals. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C),and triacylglycerol (TAG) concentrations were measured by commercial enzymatic test kits according to the manufacturer's instructions (Biomerieux, Lyon, France) using an automatic analyzer (Type 7170A, Hitachi, Tokyo, Japan).

Tissue Study

Macroscopic Assessment

The aorta was removed from the ascending aorta to the iliac bifurcation and cut longitudinally along the mid-ventral wall. Aorta was washed and cleaned by ethanol 70 and then stained with Sudan (Sigma Chemical, 15 minutes shaking). The luminal surface of each aortic specimen was photographed (under loop 20) and the image was stored electronically. Lipid strikes and red plaques were assessed by software image J.

Microscopic Assessment

Samples were embedded in paraffin blocks and stained with hematoxylin-eosin. To assess the collagen composition and possible changes, Masson's trichrome-staining was performed. Masson's trichrome-staining intensity was arbitrarily scored on a scale of four grades: 0= negative, 1= weak positivity (plaque thickness < half of media), 2= moderate positivity (plaque thickness = half of media or macrophage accumulation), 3= strong positivity (plaque thickness = thickness of media, connective tissue is presented, extra cellular matrix proliferation by smooth muscles), 4= plaque thickness > thickness of media with obvious lipid plaque (15).

Statistical Analysis

Scoring and measurements were performed by two blind independent researchers. Histomorphometric and biomechanical analyses were performed using SPSS 22 software (IBM SPSS Statistics). Scoring variables were reported as frequencies and percentages, while quantitative variables were mean ± standard deviation or standard error mean (SEM), minimum and maximum. The Fisher test for qualitative variables and the one-way ANOVA technique for quantitative variables were used. All tests were considered significant for relative values $P<0.05$, and odds ratios (OR) of 95%.

Results

Body Weights of Animals

Body weights of rabbits during the observation period, increased in all groups. However, there were no significant differences between groups ($p>0.05$) (Figure 1).

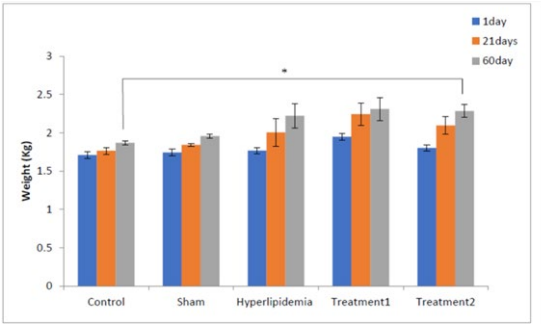


Figure 1. The effect of Boron administration on weight in all groups on days 1, 21 and 60. Data is presented as mean +_ SEM. P<0.05 is significant (*).

Group t1 received both chol 1% and Boron 4mg/kg from the first day and t2 received chol 1% and Boron starting on 20th day to the end of experiment.

Lipidemic Profile

HDL did not change in the control and sham groups while it increased within 21 days in hyperlipidemic, T1 and T2 groups (p<0.05). HDL decreased after 60 days (p<0.05). LDL did not change in the control and sham groups while it increased within 21 and 60 days in hyperlipidemic, T 1 and T2 groups (p<0.05). Cholesterol did not change in the control and sham groups during 60 days. However, it increased during 21 and 60 days in hyperlipidemic, T 1 and T2 groups significantly (p<0.05). TG did not change in the control and sham groups within 60 days,but it increased during 21 days in hyperlipidemic, T 1 and T2 groups significantly (p<0.05). TG decreased significantly (p<0.05) after 60 days in the T2 group (Figure 2).

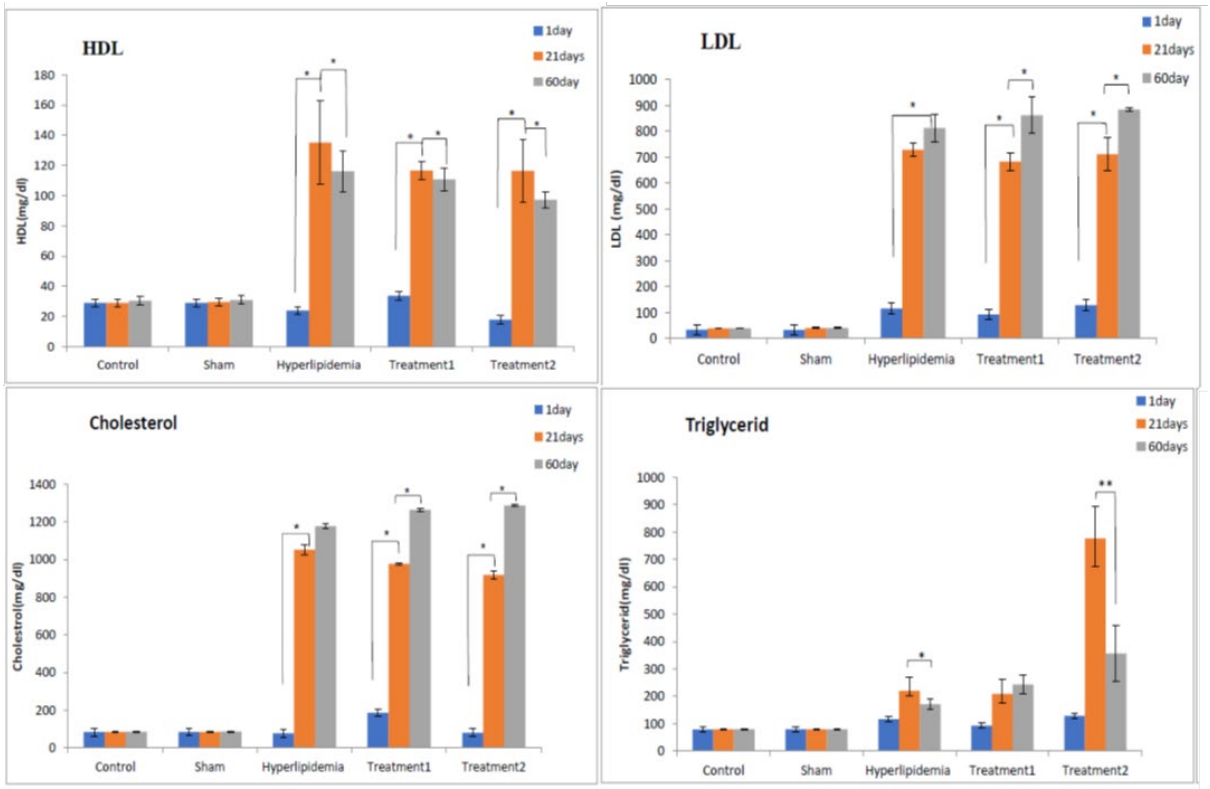
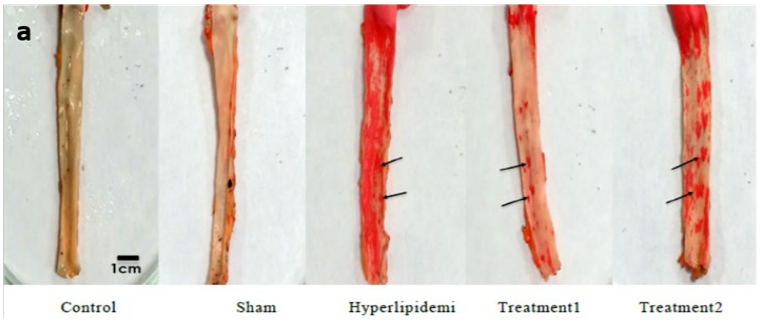


Figure 2. LDL, HDL, TG and Cholesterol increased during test time in hyperlipidemic and treatment groups (p< 0.05). Boron could reduce TG significantly after 21 and 60 days (p< 0.01). Data are shown as mean+_ SD.

Pathology

Macroscopic Study (Sudan Staining): Boron administration reduced lipid strips in the descending aorta when administered from the first day or after 21 days (Sudan staining). This was revealed when images were analyzed by Image J analysis software (78.53 % lipid strips versus 19.65 % for T1 and 37.89 % for T2 subsequently, p<0.001, Figure 3a).

Microscopic study (H & E, Trichrome staining): Microscopic study by H & E and trichrome staining after 60 days, showed that thickness of the atherosclerotic plaque increased significantly in hyperlipidemic diet. This injury was improved by boron administration in both T1 (p<0.001) and T2 groups (p<0.05) (Figure. 3b). Pathologic grading of the atherosclerotic plaque was augmented in hyperlipidemic diet and Boron significantly decreased the injury respectively (p<0.001, p<0.05, Figure. 3c). Pathologic grading was performed according to Chekanov2003 method (15).



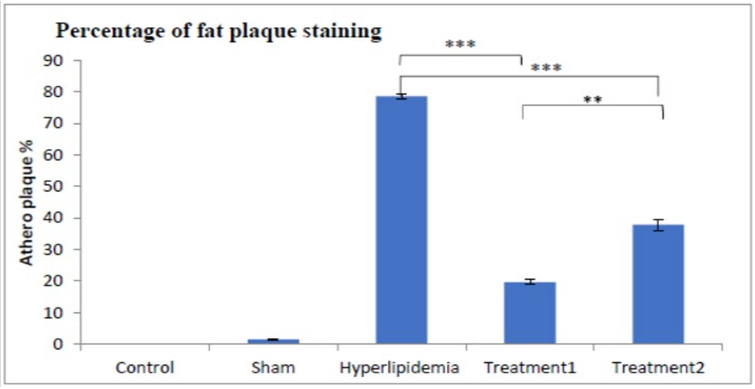


Figure 3a. Sudan staining of descending aorta showed hyperlipidemia formed 78.53 % lipid strips while diminished significantly in T1 and T2 groups (19.65 % and 37.89 % respectively, p<0.001).

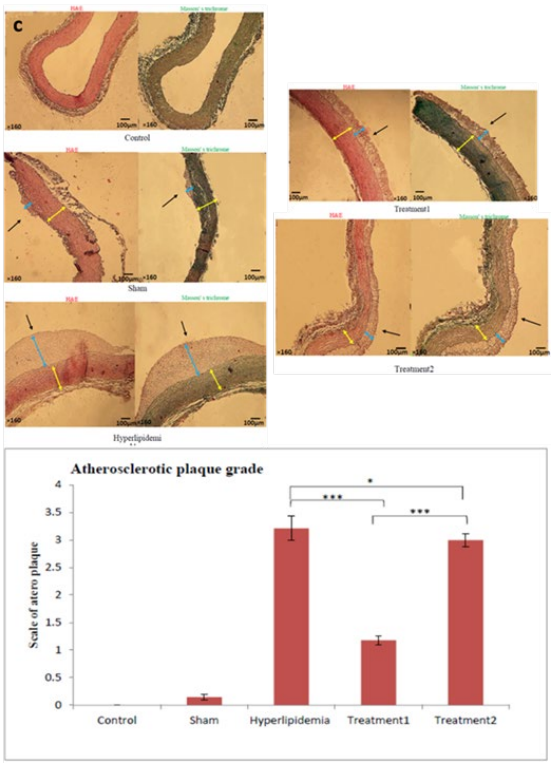


Figure 3b. Boron administration and its effect on plaque thickness in treatments 1,2 compared to the control, hyperlipidemia and sham groups. H & E and trichrome staining after 60 days. Thickness of the atherosclerotic plaque increased during hyperlipidemic diet and Boron significantly decreased the injury (p<0.05, p<0.001).

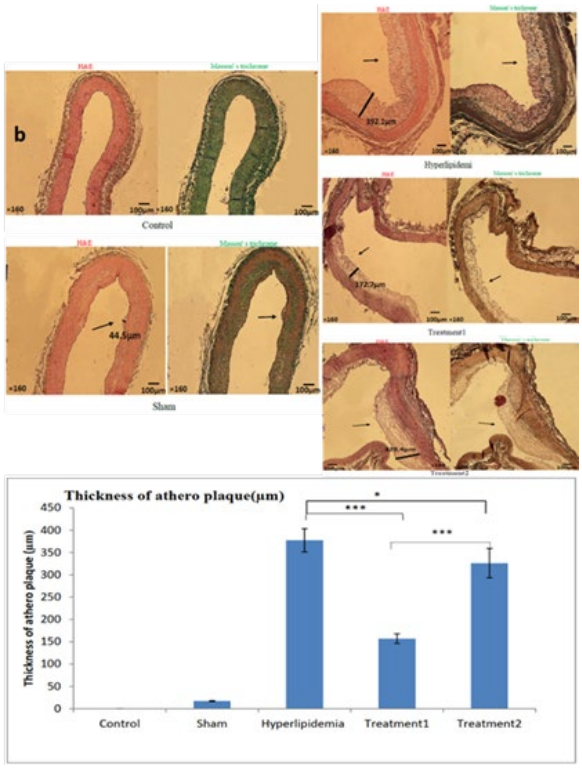


Figure.3c. Pathologic grading showed induced tissue injury after hyperlipidemia which was ameliorated with Boron treatment in T 1 and T2 groups significantly (p<0.001).

Discussion

Atherosclerosis is a progressive artery disease with subendothelial origin, and one of the main causes of cardiovascular diseases. Nowadays, antioxidants and some chemical drugs are considered to be effective

medications in prevention or treatment of atherosclerosis or other artery disorders (8). Boron is a trace element which is required for normal growth, Ca metabolism and endocrine function in the body. Boron antioxidant and anti-inflammatory properties, can contribute to atherosclerosis treatment or prevention (11).

In this study boric acid 4 mg/kg was administered to atherosclerotic animal models. Boron administration in hyperlipidemic rabbits could not prevent significant weight gain due to hyperlipidemia Study elapsed time (60 days) and cholesterol regimen intensified weight gain. Other studies showed a dose of 2 mg Boron per day, does not affect body weight gain in the short-term administration. Obviously, it seems higher doses or extended time administration of Boron may contribute to weight gain restriction. In 2015 two different studies showed 100 mg/kg and also 5 and 10 mg/kg (in diabetic rats) Boron in rats caused weight loss after 4 weeks whereas lower doses of about 2 mg/kg could not cause weight loss significantly (10). Boron administration after 21 days reduced LDL level while the effect was not obvious after 60 days of treatment through extended lipid diet. This data is consistent with Eren's data which showed Boron cannot affect serum biochemical factors such as LDL and HDL (16). Boron administration did not modify TG serum level either. In other investigations Boron at different doses (10, 20, 160 and 250 mg for 8 weeks) improved serum lipid profile which were in accordance with Naghii data (2 mg for 2 weeks) (17-19). In our study, Boron could reduce TG serum level significantly which is an important risk factor of atherosclerosis. Cakir et al., showed 10 mg Boron, when administered to diabetic rats, was more effective than 5 mg in improving serum lipid profile (10, 20). In this study, microscopic and macroscopic atherosclerotic changes were investigated too. Boron significantly reduced lipid strips especially in group 2 treatment, after 60 days of administration. For the first time it is revealed that Boron is able to prevent atherosclerosis when administered for a long time through inhibition of lipid accumulation and cholesterol reuptake from tissues. It has been claimed that Boron in the form of cis-ester with glucose or ribose act as a cofactor or regulator of cell signaling pathways. Boron is considered for its role in bone and joint health because of the effects on different steroid hormones such as estrogen, testosterone, dehydroepiandrosterone (DHEA), and 1, 25 dihydroxycholecalciferol (21). It has an important effect on the activity of various metabolic enzymes, metabolism of steroid hormones including protective role in the cardiovascular system through probable mechanisms with an emphasis on oxidative stress, fibrosis, angiogenesis, and vascular function (22). Accumulated evidence indicates that dietary Boron has a marked effect on certain metabolic processes, such as arthritis, coronary heart disease and osteoporosis (21). Boron may prevent these chronic diseases by interfering with the production of certain steroid hormones (estrogen, testosterone). Boron regulates the inflammatory response and furthermore alleviates arthritis by modifying the levels of serum antibodies and limiting T-cell activity (23). According to different reports Boron is considered as a potent anti-oxidant, anti-inflammatory and lipid metabolism regulator which can describe its beneficial effects on atherosclerosis and inflammatory diseases (19). In accordance with this hypothesis and our data, Bouchareb et al., reported that Boron has the potential to induce cardiomyocyte cell cycle entry and cardiac tissue regeneration after injury which can be a beneficial supplement in MI and a noble candidate for anti-fibrosis approach (24). Boron- based diet might increase the enzymes activity via SOD pathway (25). It is also known that extracellular SOD has protective activity against atherosclerosis, hypertension, heart failure and diabetes mellitus (26). Regarding the intervening role of oxidative stress in the development of cardiovascular damage, and noticeable Boron protective influence in oxidative events, Boron therapeutic application could be considered otherwise. Our data is in accordance with Donoiu, I. who claimed certain cardiovascular risk factors including atherosclerosis prospect may be reduced by intake of Boron compounds, leading to a lessening of cardiovascular morbidity and/or mortality (14).

Conclusion

It seems Boron could be an effective agent in prevention and treatment of atherosclerotic lesions through its anti-inflammatory and anti-oxidant protective properties.

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Conflict of Interest

There is no conflict of interest.

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