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Anti- inflammatory effect of boric acid on cytokines in ovariectomy-induced rats

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ABSTRACT

The increase in the rate of inflammation in the post-menopause period also leads to a significant increase in the use of anti-inflammatory agents**.** This study aimed to investigate the effect of BA supplementation on pro-and anti-inflammatory cytokines in ovariectomy (OVX) induced rats. A total of 48 nonpregnant female Wistar albino rats (80-100 g) were used in the experiment. Forty-eight rats were divided into six equal groups (n=8): Control, OVX, OVX+5 mg/kg BA (OVX+BA_5), OVX+10 mg/kg BA (OVX+BA_{10}), 5 mg/kg BA (BA_5), 10 mg/kg BA (BA_{10}). Serum TNF-α cytokine levels of rats in the OVX group were higher than in control rats ($P<0.05$). TNF-α levels were significantly reduced in the OVX-induced rats with 5 mg/kg BA and 10 mg/kg BA supplementation ($P<0.05$). While serum IL-1α and IL-6 levels were not different between OVX and control rats, serum IL-3 levels were low ($P<0.05$) and not affected by 5 mg/kg and 10 mg/kg BA supplementation. Serum IL-11 levels increased significantly in the OVX rats with 5 mg/kg and 10 mg/kg BA supplementation ($P<0.05$). As far as we know, certain doses (5 and 10 mg/kg) of BA are the first study on the prevention of increased inflammation in rats induced by OVX. Results suggest that the supplementation of BA regulates the inflammatory changes associated with OVX and thus has beneficial for menopause management.

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Introduction

In an aging society, the postmenopausal female population is increasing. While the total population of postmenopausal women worldwide was 477 million in 1998, it is estimated that this population will reach 1200 million by 2030. Menopause is a turn-out spot in the life of every woman, the last period of menstruation-related with the disruption of the activities of the ovarian follicle, resulting in the persistent stopping of menstruation. The onset of menopause is often related to hormone insufficiency, a factor that subscribes to a raised incidence of osteoporosis, vasomotor disorders, cardiovascular diseases and cognitive impairment. Postmenopausal period, the functioning of the ovarian follicles is decreased and ovaries produce less estrogen. Osteoporosis due to estrogen deficiency is very common in postmenopausal women, and osteoporosis occurs in 40% of postmenopausal women (1).

Tumor necrosis factor-a (TNF-α) is one of the cytokines constituting the acute phase reaction and is a cell-signaling protein involved in systemic inflammation. It is mainly synthesis by active macrophages, although CD4+ cells may be generated by many other cell types such as lymphocytes, NK cells, neutrophils, mast cells, eosinophils. The primary role of TNF-α is the regulation of immune cells (2). Interleukin 1 alpha (IL-1a) is a cytokine of the interleukin 1 family and is mainly synthesized by activated macrophages as well as neutrophils and epithelial cells. It has physiological, haematopoietic, metabolic activities, and plays a role significant role in the organizing of the immune responses. IL-1a is accountable for promoting the generation of inflammation up to fever and sepsis (3). IL-6 is a cytokine that uses the gp130 molecule in common in its multimeric complexes. The synthesis of IL-6 is mainly fibroblasts, endothelial cells and monocytes. IL-6, along with other pro-inflammatory cytokines IL-1 and TNF-α, is regarded as a significant intermediary of the inflammatory response (2, 4). Recent studies have been established the beginning of menopause is associated with a low systemic inflammatory status, an inflammation manifested through raised serum levels of the key pro-inflammatory cytokines as TNF-α, IL-1 or IL-6 (5). There is evidence that these

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increases are associated with decreases in ovarian function (6). Interleukin-3 (IL-3) excretes by the activated T cell is play an important role in the regulation of hematopoiesis (7). Interleukin-11 (IL-11) is a cytokine belonging to the IL-6 family which has anti-inflammatory potential (8).

Anti-inflammatory agents have been accepted as a valuable tool in the treatment and prevention of postmenopausal osteoporosis and inflammation. Therefore, improving new agents with anti-inflammatory action and fewer side effects for the administration of menopause continues an intriguing topic. Boron is added to many natural and new synthetic molecules that have been investigated as potential drugs recently (9). At the same time, it is added to diets (usually as boric acid: BA) to treat painful menses. Boron is a trace element commonly found in the environment (soil, water, etc.), although its plenty is low compared to other elements, it is found in plants, seeds and diverse organs of some animal species. Boron is an important role in bone metabolism and reduces the risk of inflammatory status (10, 11).

Although studies have been indicated strong evidence that OVX affects inflammatory cytokines, none of them simultaneously evaluate the relationship between pro-inflammatory and anti-inflammatory cytokines and characterize a complete cytokine profile in menopause. At the same time, there is no study evaluating the effect of trace element BA on pro-inflammatory and anti-inflammatory cytokines after OVX. This study aims to evaluate the pro-inflammatory and anti-inflammatory cytokine profile after OVX and the effect of BA on these cytokines.

Materials and methods

Animals

A total of 48 nonpregnant female Wistar albino rats (80-100 g) were used in the experiment. Rats were obtained from Aydın Adnan Menderes University (Turkey), Faculty of Veterinary Medicine, Experimental Animals unit. Before the experiment, the rats were kept 10 days for adaptation. In the adaptation and experimental periods, rats were kept in polyethylene cages under the same standard conditions (light/dark cycle of 12:12 h, at 21±2°C with 55-60% humidity). The study was approved by the local ethics committee convened by the Animal Care and Use Committee of Aydın Adnan Menderes University and was performed in accordance with animal protection regulations (Ethics number: 64583101/029).

Experimental design

Rats were divided into six groups, each containing eight rats: control (Group 1), ovariectomy (Group 2, OVX), ovariectomy + 5 mg/kg boric acid (Group 3, OVX+BA_5), ovariectomy + 10 mg/kg boric acid (Group 4, OVX+BA_{10}), 5 mg/kg boric acid (Group 5, BA_5), 10 mg/kg boric acid (Group 6, BA_{10}). Rats were ovariectomized under ketamine anesthesia. Anesthesia was confirmed by a decreased respiratory rate and unresponsiveness. Abdominal incisions were ejected the lateral skin of rats and ovaries were removed. Ovaries were isolated by ligation of the closest part of the oviduct. The analogous procedure was performed on rats in the control group, except that the wound was closed without removed ovaries (12). OVX was performed on rats in the 2nd, 3rd and 4th groups, and controlled OVX was performed on rats in the 1st group simultaneously. No operation procedure was applied to rats in the fifth and sixth groups. Since it was reported that bone loss starts 2 weeks after OVX in rats and the most intense loss was observed within 100 days, BA supplementation was started 2 weeks after the operation. Rats were given BA (Sigma-B0252, USA) for 20 days by oral gavage. Since there was no consensus in studies with BA dose and OVX, two references values as BA dose was determined as 5 mg/kg and 10 mg/kg considering the data of WHO in 1998 and other studies (13). Rats in the third and fifth groups were given by oral gavage method daily 5 mg/kg BA, while fourth and sixth groups were given by oral gavage method of daily 10 mg/kg BA. During this period, BA was not applied to the first and second groups. At the end of the 21st day, blood samples were collected from rats under ketamine anesthesia by cardiac puncture (14). After total exsanguination by cardiac puncture under general anesthesia, the collected blood was centrifuged, and the serum was stored at - 80°C until analyses.

Measurement of pro-inflammatory cytokines

Changes of pro-inflammatory cytokines in serum samples were assessed via specific ELISA kits, according to instructions. To evaluate their action of

pro-inflammatory cytokines, TNF-α (eBioscience Rat TNF-α Platinum ELISA kit, Austria), IL-1α (eBioscience Rat IL-1α Platinum ELISA kit, Austria), IL-3 (Cusabio Rat IL-3 ELISA kit, ABD), IL-6 (eBioscience Rat IL-6 Platinum ELISA kit, Austria) and IL-11 (Cusabio Rat IL-11 ELISA kit, ABD) used. Cytokines levels were expressed as pg/ml.

Statistical analysis

The data were statistically analyzed using the SPSS (SPSS Inc. version 24, Chicago, IL, USA) statistical program. The Shapiro-Wilk test was performed to verify data normality. Differences between means of different groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test comparisons. Results were exhibited as the mean ± standard error of the mean (x ± SEM). $P<0.05$ was considered statistically significant.

Results and discussion

Serum TNF-α, IL-1α, IL-3, IL-6 and IL-11 cytokine levels result for rats included in the study displays in Figure 1. Serum TNF-α cytokine levels of rats in the OVX group were significantly higher than in the control rats ($P<0.05$). The OVX+BA_5 and OVX+BA_{10} rat groups were reduced significantly the level of TNF-α ($P<0.05$). TNF-α cytokine levels in the BA_5 and BA_{10} supplementation rats were not different from the control rats. Serum IL-1α and IL-6 cytokine levels were not different among the control, OVX and BA supplement rats ($P>0.05$). Serum IL-3 cytokine levels were statistically higher in the control, BA_5 and BA_{10} rats compared to OVX rats ($P<0.05$). Serum IL-3 cytokine levels in OVX+BA_5 and OVX+BA_{10} rat groups were not different than OVX rat groups ($P>0.05$). Although serum IL-11 cytokine levels in the OVX rats were higher than control rats, there was no statistical difference ($P>0.05$). Serum IL-11 cytokine levels were significantly higher in the OVX+BA_{10} and BA_5 rats than in the other rat groups ($P<0.05$).

Studying molecular alters related to menopausal status in humans is difficult because of restricted reach to tissue and serum samples and internal variation between patients. The menopause model induced by bilateral OVX in rats has been extensively used to research test new interventions and mechanisms capable of minimizing the harmful impact related to menopause (5, 15, 16). In OVX rats and postmenopausal women experience increased bone resorption and an overall decrease in bone mass in relation to estrogen deficiency. However, when investigating the problems associated with bone loss due to hormonal deficiency, osteoporosis is not only associated with hormonal loss, but also with inflammatory deterioration associated with aging (17).

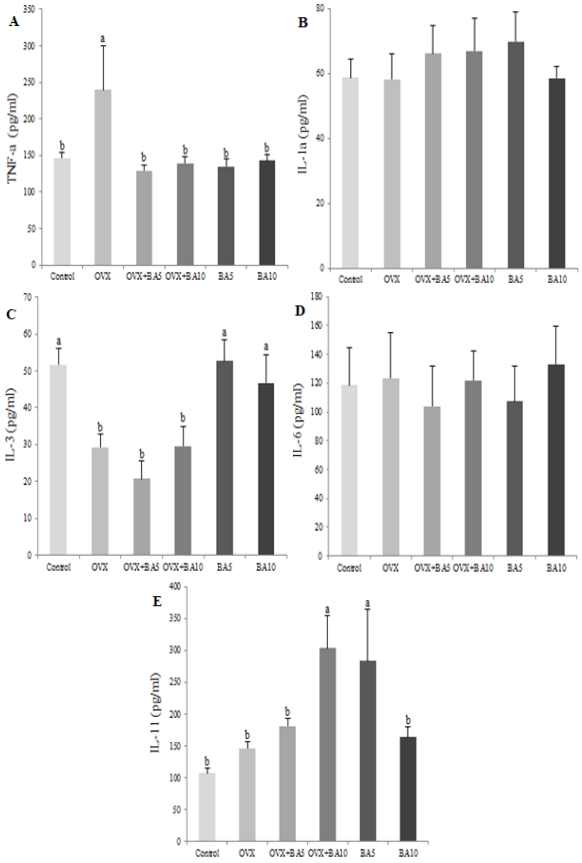

Figure 1. Effects of BA on the concentration of serum TNF-α (A), IL-1α (B), IL-3 (C), IL-6 (D) and IL-11 (E) cytokines levels of the female rats. Values are shown as mean ± standard error of the mean (x ± SEM). Different lowercase letters (a, b) indicate a statistically significant difference in groups ($P<0.05$).

Especially, the prevalence of inflammatory diseases and metabolic syndrome increases with the excessive production of pro-inflammatory cytokines due to the decrease in estrogen during menopause (6). Compston (2001) indicated that a relationship between inflammatory cytokines and estrogen, and indicated estrogens decrease bone resorption by directly inhibiting osteoclasts and indirectly by depressing osteoblastic production of some pro-inflammatory

factors (18). Previous studies have been confirmed that levels of pro-inflammatory cytokines are found to be high in postmenopausal women (19). Studies on pro- and anti-inflammatory cytokines have been increased in recent years, and many cytokines have been revealed to have important effects on the immune and hematological systems (4, 5).

The inflammatory immune response occurs through activation of immune cells which secreted pro-inflammatory cytokines, such as TNF-α, IL-1α and IL-6. Experimental and clinical studies have been indicated that TNF-α, IL-1α and IL-6 are cytokines associated with infection, trauma, and sepsis syndrome (4, 20). TNF-α starts and, regulates cytokinesis cascade during the inflammatory response. TNF-α is known to be a pleiotropic inflammatory cytokine associated with various biological processes (21). Besides regardless of pathological conditions, in the presence of high TNF-α, a normally paired absorption/formation system important for bone homeostasis is disrupted and directly raised the activity of mature osteoclasts (7). Das et al. (2002) reported that TNF-α is elevated in healthy premenopausal women undergoing OVX and reaches its highest levels 8 weeks after OVX (22). In this study detected that serum TNF-α levels were increased in the OVX rat groups than in control rats. Similarly consistent with our findings, earlier studies previously studies have been reported an increased release of TNF-α post-OVX (4, 16). This increase can be associated with impaired monocyte and macrophage function due to estrogen lack. In addition, decreases in estrogen levels after menopause indicates increased systemic inflammation accompanied by increased synthesis of pro-inflammatory cytokines such as TNF-α (23). So the study supports the hypothesis that TNF-α exerts a pro-inflammatory effect after menopause.

IL-1 is a significant initiator of immune reply and plays a role in the development and start of a complicated inflammatory cascade. It supports bone resorption in vitro and reasons hypercalcemia and bone loss when infused in vivo (3). Lee et al. (2020) reported increased serum IL-1 levels in the OVX rats than control rats (24). Another study demonstrated that IL-1 levels were higher in bone marrow cultures of early postmenopausal women than premenopausal women, but there was no difference between premenopausal women and late postmenopausal women (25). Elder et al. (1996) indicated IL-1 mediates only a significant section, but not all, of TNF-α-induced osteoclast formation in inflammatory osteolysis (26). In the present study, IL-1α levels were not increased in the OVX rats according to control rats and were not accompanied by increased TNF-α levels. IL-1 findings of this study are consistent with by Hustmyer et al. (1993), Elder et al. (1996) (26, 27). TNF-a and IL-1a cytokines are potent inducers of IL-6 cytokine, which is involved in the transformation of osteoclast precursor cells into mature osteoclasts. IL-6 is a multifunctional cytokine in host defense and primarily modulates inflammatory responses (28). Nanashima et al. (2020) reported that IL-6 increased osteocyte-mediated osteoclast differentiation and osteoclastogenesis (29). Another study detected that pro-inflammatory serum marker, IL-6 raised after menopause in healthy women (30). In this study, there was no difference in serum IL-6 levels between OVX and control rats similar with IL-1α results, and these findings are in agreement with the study results of Bruunsgaard and Pedersen (2003) (17). Researchers showed that IL-1α stimulates IL-6 production, and IL-6 affects IL-1α synthesis and stated that these cytokinesis levels are directly related to each other. At the same time, it is thought IL-1a antagonist IL-1ra or anti-IL-6 antibodies may have blocked the functional activity of IL-1 and IL-6 cytokines in mature animals by infusing specific inhibitors (31). Our study data does not support the pro-inflammatory effect of IL-1a and IL-6 cytokines after menopause. IL-3 acts on many target cells, including mast cells and macrophages. Yogesha et al. (2009) stated that IL-3 inhibited both osteoclastogenesis and bone resorption induced by TNF-α and other pro-inflammatory cytokines (7). Another study showed that IL-3 has a pro-inflammatory effect on human osteoclast cell differentiation (32). In this study, serum IL-3 levels were lower in the OVX rats than control rats, indicating that not support the pro-inflammatory effect of IL-3 after menopause, as the serum levels are lower compared to the genital activity period. Also, there is currently no study in the literature in which we can compare changes in serum IL-3 levels after OVX. Therefore, this study data should be approached with precaution. More studies are needed to determine the effect of IL-3 in the post-menopausal period.

IL-11 is a cytokine with an anti-inflammatory effect. Anti-inflammatory cytokines are a set of immune regulators that control the pro-inflammatory cytokine response. Indeed IL-11 has impacted many bioactivities such as inducing immunoglobulin production and stimulating the acute phase response (2). IL-11 applies its anti-inflammatory impacts by directly reducing the release of inflammatory agents such as nitric oxide (NO), TNF-α, IL-1 and IL-12p40; also may antagonize TNF-α by induction of soluble TNF-RI (8). In addition, it prevents nuclear factor κB (NF-KB) translocation by increasing the expression of NF-kB (IκB) inhibitors. Because TNF-α and IL-1b promoters have NF-κB binding sites the IL-11 might downregulate pro-inflammatory cytokines (33). In the current study, although serum IL-11 levels were high in the OVX rats, there was no statistical difference with control rats. However, increasing IL-11 levels to antagonize the increased TNF-α effect in the OVX rats reveals the anti-inflammatory effect post-OVX(34).

In the effort to produce new drugs that alleviate inflammation and the osteoporosis process in the postmenopausal period, much attention has been put to the search for natural molecules due to their availability, cost and biological activities. Avsar et al. (2013) reported that *Panax Ginseng* supplementation decreased serum TNF-α, IL-1b and IL-6 levels in the OVX rats (35). Huyut et al. (2020) detected that zaprinast and avanafil supplementation decreased IL-1β levels in the OVX rats, but did not affect IL-6 and TNF-α levels (36). Another study showed that cod bone gelatin treatment can positively affect bone improvement by suppressing the release of TNF-α, IL-1 and IL-6, which often increases post-OVX (37). Many studies revealed the anti-inflammatory effects of curcumin, vanillic acid, soybean, geraniin, vicenin in the OVX rats (5, 16, 20). In this study, we used BA to test the anti-inflammatory effect against the inflammatory changes associated with OVX. BA has hepatoprotective, antioxidant, anti-inflammatory and immunomodulatory activities in animals (11, 38-41). Dietary boron intake has been suggested to have immunostimulatory effects, including the proliferation of T cells and enhancement of natural killer (NK) cell function in different organisms (11). Naghii et al. (2011) stated that a significant decrease in plasma TNF-α concentration after one week of boron supplementation in healthy humans and, boron supplementation was effective in reducing inflammatory biomarker levels (42). The increasing number of research have shown that boron decreases levels of inflammatory biomarker and modulates cellular reply in inflammatory processes (9, 10). But literature reviews indicated that there have been no studies investigating the effect of supplementation BA on pro-inflammatory and anti-inflammatory cytokines in the OVX rats. In the current experimental study, in OVX-induced rats decreased supplementation of 5 mg/kg BA and 10 mg/kg BA significantly TNF-α levels. This decrease is considered to be closely associated with the anti-inflammatory effect of BA. However, IL-1α, IL-6 and IL-3 cytokine levels which are other pro-inflammatory markers, were not affected by supplementation of 5 mg/kg and 10 mg/kg BA to OVX rats. The reason for this may be related to the dose and duration of BA supplementation. Therefore, for these cytokines, higher doses of BA supplementation may be recommended as replacement therapy. IL-11, known as an anti-inflammatory cytokine, increased significantly in the OVX rats with 5 mg/kg and 10 mg/kg BA supplementation. The increased IL-11 anti-inflammatory cytokine levels by dose-dependent treatment of BA, indicating that BA has an anti-inflammatory effect. An antagonistic relationship between IL-11 and TNF-α cytokines was observed in BA-treated OVX rats. As far as we know, this information will enter our work for the first time in the literature. Our study revealed that inflammation occurred in rats induced by OVX. BA exerted protective effects against inflammation in post-OVX rats, possibly by manipulating cytokines which are an indicator of inflammation. The usability and potential side effects of BA in the OVX rats should be well-defined. In conclusion, this study stated that inflammation occurs in rats by OVX, and revealed the supplementation of BA regulates the inflammatory changes related to OVX and thus has beneficial for menopause management.

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Conflict of interest

The authors of this article have no conflict of interest.

Author's contribution

HT analyzed the serum samples, prepared the table, interpreted the data, searched the literature and prepared the manuscript. AB designed and coordinated the study. GA analyzed the serum samples. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version

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