Do Avanafil and Zaprinast Change Some Selected Cytokine Levels In Ovariectomized Rat's Liver?

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ABSTRACT

Studies reported that phosphodiesterase-5 inhibitors (PDE-5Is) positively contributed to bone-mineral-density and thickness in rats with ovariectomy, which have the same condition with postmenapozal period. To explain the positive contribution mechanism on bone mineral density of PDE-5Is, we investigated the effect of zaprinast and avanafil on levels of some pro-or anti-resorptive cytokines in ovariectomized-rats.

Albino female rats (8 months and 250-350 g) were used and four groups of equal-number were randomly assigned (n=6). Groups; was the sahm operated, positive control (OVX), Zaprinast and OVX, Avanafil and OVX groups, respectively. The levels of Estrogen, IL-1β, IL-6, IL-8, IL-10 and TNF-α were measured by ELISA kits, in liver of rats.

IL-1β, IL-6, IL-8 and TNF-α levels were high in groups with OVX compared to sham group, while IL-10 levels were low. Also, IL-1β, IL-6, IL-8 and TNF-α levels were low in zaprinast and especially avanafil-treated groups with OVX and were similar to the sahm group values (p=0.001 for IL-1β, p=0.045 for IL-6, p=0.008 for IL-8, p=0.006 for IL-10, p=0.026 for TNF-α).

Zaprinast and especially avanafil inhibited IL-1β, 8 and TNF-α and increased the IL-10 levels compared to the OVX group. This may support opinion that PDE-5Is enhance bone mineralization by inhibiting proresorptive cytokines.

Key Words: Avanafil, phosphodiesterase-5 inhibitors, cytokine, ovariectomy, zaprinast

Introduction

Decreasing estrogen levels after menopause degrade bone turnover in favor of bone destruction, resulting in postmenopausal osteoporosis. One of the ways to reflect the postmenopausal period experimentally is ovariectomy, which causes estrogen deficiency. Although anabolic activity of the bone increases after ovariectomy, bone resorption becomes more dominant (1).

The studies of both in vivo in rodents and in vitro showing the association of pro-inflammatory cytokines in the pathogenesis of bone resorption reported to play a critical role in bone remodeling mechanism (2). High serum proinflammatory cytokines, cytokine soluble receptors and C-reactive protein levels are associated with increased fracture risk (3). Prorezorptive cytokines such as tumor growth factor beta (TNF-α), interleukin (IL) 1β, IL 8, 6, 12, 7 and 20 stimulate osteoclastogenesis and bone resorption, while antirezorptive cytokines such as tumor growth factor beta (TGF-β), IL 4, 10, 12, 13, 23, 33 and interferon-gamma (IFN-γ), have been reported to have opposite effects (4,5). In humans, high proinflammatory cytokines have been associated with increased fracture risk and bone loss (2). There is a significant positive correlation between IL 1β and TNFα in women with osteoporosis. TNFα and IL 1β are known to inhibit bone formation by suppressing osteoblast activity (6).

Sildenafil from phosphodiesterase-5 inhibitors (PDE-5Is) has been reported to reduce upregulation of pro-inflammatory cytokines such as TNFα and IL 1β (7). In addition, Ahmet *et al.* demonstrated that sildenafil reduced the production of proinflammatory cytokines such as TNFα and IL 1β (8).

In a previous our study, we showed that zaprinast and avanafil from PDE-5Is contributed positively to bone mineral density in male rats with dexamethazone-induced osteoporosis (9). In another study of our group, the PDE-5Is such as vardenafil, udenafil and tadalafil were shown to have a positive effect on bone mineral density in

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ovariectomized-rats (10). In these studies, the positive effects of PDE 5 inhibitors on bone density were found to be mediated by the angiogenesis and NO/cGMP/PKG pathway. In addition to this mechanism, PDE-5Is may have performed this positive effect on bone mineral density by inhibiting antiresorptive cytokines in different tissues. However, there are few studies investigating the effect of PDE-5Is on pro or anti-resorptive cytokines. According to our exploration, the effects of zaprinast and avanafil have not been investigated on liver tissue TNFα, IL 1β, 6, 8 and 10 levels, which are known to be important in both bone metabolism and tissue inflammation.

This study focused the effect of zaprinast and avanafil on the levels of IL-10 from antiresorptive cytokines and IL 1β, 6, 8 and TNF-α from proresorptive cytokines in liver tissue of ovariectomized rats. In this way, it was aimed to clarify whether another mechanism mediated to the positive effect of their on bone mineral density by investigating effect of zaprinast and avanafil on these cytokines.

Materials and Methods

Chemicals: The PDE-5 inhibitor, zaprinast were purchased from Sigma (Catalog number: Z0878) (Sigma Aldrich, USA). PDE-5 inhibitor, avanafil (CID: 330784-47-9) was purchased from PubChem. Enzyme linked immunosorbent assay (ELISA) kits were purchased commercially (YL biont, Shanghai YL Biotech Co., Ltd).

Ethical Approval and Experimental Design: In this study, 24 female wistar-albino rats (eight month old and 250-350 g) were used. This study was approved by Experimental Animals Local Ethics Committee of Van Yuzuncu Yıl University, and all procedures were performed in accordance with the ethical rules stated in Helsinki Declaration in 2000. During the study, the rats were fed with standard pellet feed in dark and light room conditions for per day at 12 h. The groups were generated as follows;

Sham Group: Under anesthesia, abdominal areas of rats were opened approximately 2 cm and closed again. After postoperative care, rats were fed with standard pellet feed for 8 months. At the end of this time, intracardiac blood and other tissues were taken under anesthesia.

OVX Group: Under anesthesia, the abdomen regions of rats were opened approximately 2 cm and the ovaries were removed and closed again.

After postoperative care, rats were fed with standard pellet feed for 8 months. At the end of this time, intracardiac blood and other tissues were taken under anesthesia.

OVX+Zaprinast Group: Under anesthesia, abdomen regions of experiments were opened approximately 2 cm and ovaries were removed and closed again. After feeding for 6 months with standard pellet feed, daily 10 mg/kg zaprinast was given intraperitoneally in addition to the standard feed for 2 months. At the end of this time, intracardiac blood and other tissues were taken under anesthesia.

OVX+Avanafil Group: Under anesthesia, the abdomen regions were opened approximately 2 cm and the ovaries were removed and closed again. After feeding for 6 months with standard pellet feed, daily 10 mg/kg avanafil was given intraperitoneally in addition to standard pellet feed for 2 months. At the end of this time, intracardiac blood and other tissues were taken under anesthesia.

Plasma Samples: At the end of study, the rats were anesthetized and euthanized. Intracardiac blood samples and liver tissues were taken. The whole blood in hemogram tube was centrifuged at 2500 ×g for 15 min and the plasma samples were divided and stored at −80°C.

Measurement of Estrogen Levels in Plasma Samples: Plasma estrogen levels were measured with ELISA (Eastbopharm) kits in accordance with the kit prospectus. The results were expressed in ng/L.

Homogenization of Liver Tissues and Supernatant Production: 1.8 mL of phosphate buffer (50 mM and pH: 7.4) was added to 0.2 g of tissue. The mixture was homogenized with the homogenizer (Ultra Turrax-T25) for about 15 seconds. After centrifugation at 3000 *xg* for 20 minutes, biochemical parameters were studied in the liver supernatant.

Measurement of Selected Cytokine Levels in Liver Supernatant: TNFα, IL 1β, 6, 8 and 10 levels in liver tissue homogenates were evaluated in using the commercial kits by an enzyme linked immunosorbent assay (ELISA, YL biont, Shanghai YL Biotech Co., Ltd) method.

Statistical Analysis: SPSS 22 (Inc, Chicago, Illinois, USA) was used for statistical analysis. Descriptive statistics of the groups were expressed as mean and standard deviation. Kruskal-Wallis test was used to determine whether the differences between the groups were significant within the same parameter. Post-hock was performed to
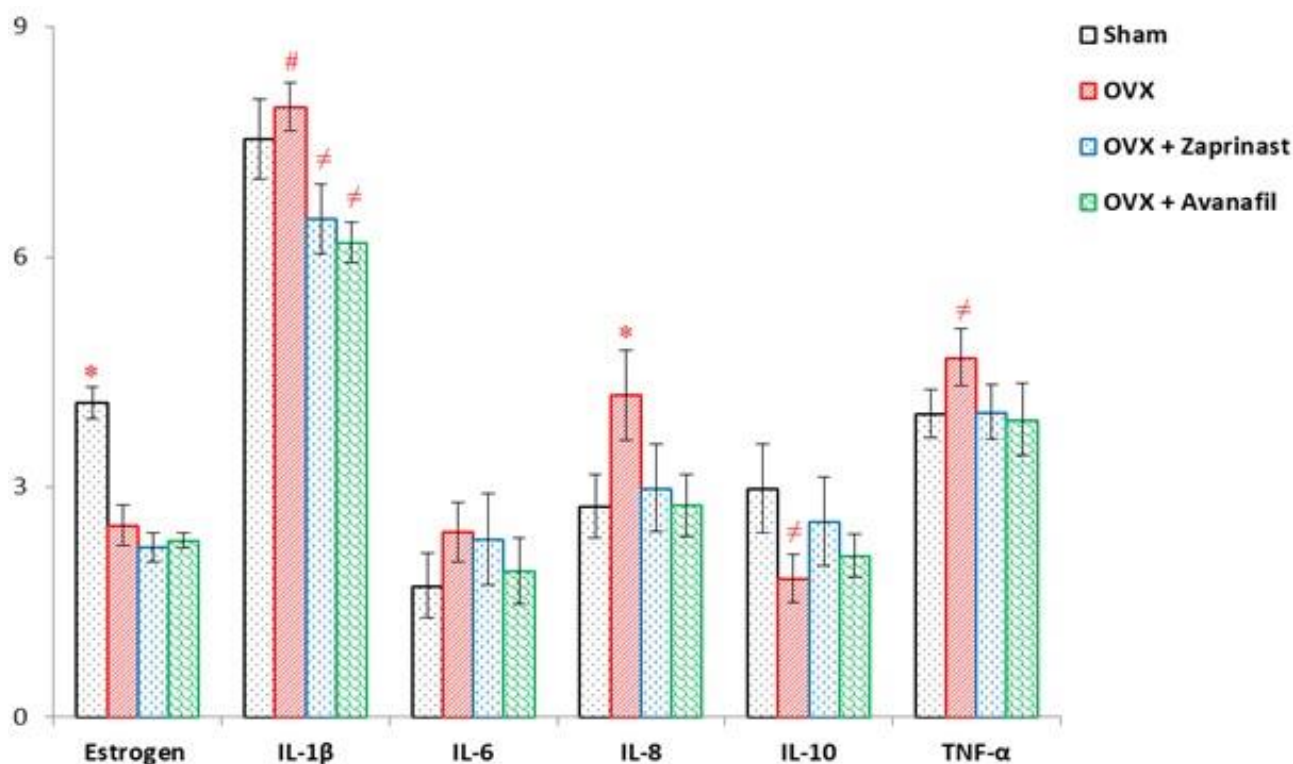
Fig. 1. The figure shows the effects of zaprinast and avanafil on some cytokine levels in liver of female rats with ovariectomy. Estrogen: ng/dL, IL-1β: pg/0.1 g tissue, IL-6: pg/0.01 g tissue, IL-8: ng/g tissue, IL-10: ng/g tissue, TNF-α: ng/g tissue, *p: compared with the other groups (p<0.05), ≠p: compared with the sham group (p<0.05), #p: compared with the OVX+zaprinast and OVX+avanafil groups (p<0.05).

investigate that the significant differences are from which group. P values less than 0.05 were considered to be significant.

Results

Plasma estrogen level in the all groups of ovariectomized-rats was dramatically lower than in the sham group (p=0.001). However, there was no significant difference between the ovariectomized positive control (OVX) and OVX+inhibitor groups (Figure 1, p> 0.05).

The average levels of TNFα, IL 1β, 6, 8 and 10 and comparison of within groups are shown in the Table 1 and Figure 1. In the OVX group, IL 1β levels were higher than in the sham and OVX+inhibitor groups (p=0.001). However, this difference was not significant compared to the sham group (p>0.05). In addition, IL 1β levels in OVX+inhibitor groups were low compared to both sham and OVX groups (p=0.001).

IL 6 levels of the all groups were similar to each other and there was no significant difference between them (p>0.05). In addition, IL 8 levels in the OVX group were higher than in the sham and OVX+inhibitor groups (p=0.045). There was no

significant difference between the other groups (p>0.05).

In addition, TNFα levels in the OVX group were higher than in the other groups (p=0.026). However, this difference was not significant compared to the sham group (p>0.05). Also, TNF-α levels in the OVX+inhibitor groups were lower than in the OVX group (p=0.026).

When IL 10 levels were compared, IL 10 levels in the OVX group were lower than in the other groups (p=0.006), while they were unchanged in the sham and OVX+inhibitor groups and each other were similar (p> 0.05).

Discussion

Previous studies reported that natural or surgical menopause is associated with a release of TNFα and IL 1β from peripheral blood monocytes. This effect was reversed by estrogen replacement. In this respect, previous studies in rats suggested that were need a combined blockade of both TNFα and IL 1 in order to completely prevent bone loss after ovariectomy (11).

Estrogen deficiency is resulted in a slight increase in the production of proinflammatory cytokines such as IL 1β, 6, 7 and TNFα, which support bone

Table 1. Mean and standart deviation values belonging to the cytokines levels in liver homogenates of the all experimental groups

Groups	IL 1β (pg/g tissue)	IL 6 (pg/g tissue)	IL 8 (ng/g tissue)	IL 10 (ng/g tissue)	TNFα (ng/g tissue)
Sham	75.43 ± 5.2	171.6 ± 42.2	2.75 ± 0.41	2.99 ± 0.58	3.96 ± 0.32
OVX	79.58 ± 3.2#	241.3 ± 38.8≠	4.20 ± 0.59*	1.81 ± 0.32≠	4.69 ± 0.37*
OVX+Zaprinast	64.97 ± 4.6≠	232.1 ± 59.9≠	2.99 ± 0.57	2.56 ± 0.58θ	3.98 ± 0.36
OVX+Avanafil	61.90 ± 2.6≠	190.5 ± 43.1	2.76 ± 0.41	2.11 ± 0.28	3.88 ± 0.47
p values	0.001	0.045	0.008	0.006	0.026

*p: compared with the other groups (p=0.008 for IL-8-α, p=0.026 for TNF-α), ≠p: compared with the sham group (p=0.001 for IL-1β-α, p=0.045 for IL-6, p=0.006 for IL-10), #p: compared with the OVX+zaprinast and OVX+avanafil groups (p=0.001),), θp: compared with the OVX+zaprinast group (p=0.006)

resorption, and increasing osteoclastogenesis in bone marrow (12). In one study was reported that estrogen inhibited significant release of proresorptive cytokines such as TNFα, IL 1β, 6, 8 and 17 as well as suppress the receptor activator of nuclear factor kappa B (NFkB) (4).

IL 6 is vital for fracture healing. Accumulated evidence confirmed that IL-6 is associated with cartilage destruction in rheumatoid arthritis, differentiation of preosteoblasts and apoptosis, and mineralization and dulling of the fracture callus (13). Al-Daghri et al., found in osteoporosis group that proinflammatory cytokines (IL 1β and 6) were significantly higher than the controls consistenly with other studies investigated the relationship between osteoporosis and inflammation (6).

IL 10 is a strong immunosuppressant. Experimental studies confirmed the anti-inflammatory effect of IL 10 in IL 10 deficient mice. It has been reported that trabecular separation is even greater in IL 10 deficient mice (14). In previous a studies, it has been rerported that doubling of IL 10 levels corresponds to a 20% reduction in vertebral fracture risk (15). One study showed that the concentration of IL 8 was significantly higher in the postmenopausal osteoporotic women compared to the control group and the IL 10 concentration was lower than in the control group (16).

In our study, plasma estrogen levels were dramatically lower consistent with previous studies (11,17) in ovariectomized rats. In addition, the levels of IL 1β, 6, 8 and TNFα were significantly higher in the OVX group with low estrogen level. However, IL-10 levels were significantly lower in the same group. These results are consistent with the reports of previous studies (4,12). However, in the groups with zaprinast and avanafil, IL 1β levels were lower than the OVX group whereas IL 10 levels were preserved and similar to the control levels.

Şentürk et al. demonstrated that the phosphodiesterase-5 inhibitor tadalafil improved levels of IL 6 and TNFα in rats with experimental spinal trauma, and improved functional neurological recovery (18). Also, Yıldırım et al. and Ahmed et al. showed that phosphodiesterase-5 inhibitor sildenafil suppressed upregulation of TNFα and IL 1β in different disease models (7,8). In this study, zaprinast and especially avanafil decreased IL 1β and 8 levels significantly compared to the OVX group. In addition, zaprinast and avanafil showed an important supressing effect on IL 6 and TNFα levels but this was not significant.

The zaprinast and avanafil may have a positive effect on BMD by suppressing the pro-resorptive cytokines such as IL 1β, 6, 8 and TNFα. Also, they can activate antiresorptive stocks such as IL 10. However, in order to find the exact answer to the question of how zaprinast and avanafil make positive contributions to bone mineral density in ovariectomized rats;

1- It is important to study together with the bone morphogenic proteins (BMPs) such as BMP 2, 4, 7, 9 and 13 as well as the other proresorptive and antiresorptive cytokines in both liver and bone tissues.

2- The possible effects of PDE-5Is on teriparatide, which have positive effects on bone tissue, and dickoff-related protein (Dckk-1) levels that supress osteoblastic activity, should be studied.

3- In addition, their possible effects on osteoblast apoptosis factors such as Bax, Bcl2, caspase 8, 3 and 9 should be investigated.

with opportunities from the Department of Biochemistry, Faculty of Medicine, Van Yuzuncu Yıl University and Ataturk University.

Conflict of Interest: The authors have no conflicts of interest associated with publication of this article.

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