ORIGINAL ARTICLES

Effect of linseed oil, fish oil and alendronate sodium on ovariectomy-induced osteoporosis in female rats

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ABSTRACT

Estrogen deficiency in postmenopausal women resulted in increased production of several osteoclastogenic cytokines as IL-1, IL-6 and TNF-α. Poly unsaturated fatty acids (PUFAs) are thought to have an effect on estrogen deficiency-induced osteoporosis through their anti-inflammatory activity. This study evaluated the effect of linseed oil (LO), fish oil (FO) compared to alendronate sodium on bone mineral density and serum bone turn over markers in ovariectomized rats. Rats were rendered osteoporotic by bilateral ovariectomy and maintained on modified diet for 12 weeks. Linseed oil (90, 180 mg/kg po), fish oil (90, 180 mg/kg po) and alendronate sodium (3 mg/kg po) were administered to osteoporotic female rats for 8 weeks. Dual-energy X-ray absorptiometry (DEXA) measurements for femur bone mineral density showed that all the administered drugs resulted in significantly lower bone loss among ovariectomized rats after 8 weeks of treatment. Also serum biomarkers revealed that all the given drugs significantly decreased bone formation marker osteocalcin (OCN), decreased bone resorption markers tartrate-resistant acid phosphatase (TRAP) activity and decreased serum TNF-α. These results indicate that plant source and animal source of dietary n-3 PUFA can reverse bone metabolism via a decrease in bone resorption and increase in bone formation.

Key word: ovariectomy, linseed oil, fish oil, alendronate sodium, osteoporosis.

Introduction

Osteoporosis is a condition of decreased bone mass that is prevalent in postmenopausal women and places them at risk of fractures. In female rats, ovariectomy artificially induces a marked reduction in endogenous estrogen concentrations subsequently causing a negative bone remodeling balance that augments bone loss and increases the incidence of osteopenia (Shiraishi et al., 2009). During menopause, the osteoprotective effect of estrogen is compromised due to diminishing levels of this hormone that lead to elevated secretions of bone-resorptive agents such as interleukin (IL)-1, tumor necrosis factor-α (TNF-α) and prostaglandins (prostaglandin E2 [PGE2]), which are strong promoters of osteoclastogenesis and osteoclastic activity (Fernandes et al., 2003).ALA is an essential n-3 polyunsaturated fatty acid (PUFA) which cannot be synthesized by the body due to a lack of the delta-15 desaturase enzyme and must be obtained from the diet. Upon ingestion and absorption, ALA is mainly beta-oxidized as fuel in various tissues while smaller amounts are accumulated into tissues, used for lipid synthesis or excreted (Vos et al., 2003).Linseed oil contain 56% ALA that can be converted to longer chain fatty acids estimated to be between 0.2-6% eicosapentaenoic acid (EPA) and to less than 0.05% docosahexaenoic acid (DHA) through the action of the delta-6 and delta-5 desaturase enzymes (Burdge et al., 2002). The specific fatty acids for fish oil EPA (20:5 n-3) and DHA (22:6 n-3) are homologues to arachidonic acid produced by omega 6 (20:4 n-6) which inhibit arachidonate metabolism in the cyclo-oxygenase pathway resulted in reduction of pro-inflammatory and pro-thrombotic n-6 eicosanoids. In addition, n-3 LC PUFA can suppress production of pro-inflammatory cytokines and cartilage degradative enzymes (Kew et al., 2004).

Alendronate is a nitrogen containing bisphosphonate, comprised of inorganic pyrophosphate analogue (P-O-P) where the germinal oxygen has been substituted by carbon (P-C-P) to prevent it from inactivation in gastrointestinal tract by mucosal border of phosphatases (Vasikaran, 2001) and to increase its affinity to the hydroxyapatite component of bone and localizes these compounds to the target tissue (Rosen et al., 1996). Alendronate, like other bisphosphonates, is a bone resorption inhibitor being used in the prevention and treatment of bone diseases (Shiraishi et al., 2009).

The aim of this study was to evaluate the effect of linseed oil, fish oil compared to alendronate sodium on ovariectomy-induced osteoporosis in rats.

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Material and Methods

Animals:

Female albino Wistar rats weighing 130-150 g obtained from the animal house of the National Research Center (Dokki, Giza, Egypt), were used for all experiments of this study. Experiments were performed according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC). Animals were housed 8-10 per cage at room temperature, under constant conditions of light and humidity, throughout the period of experimentation. The animals were fed a modified diet contain concentrated animal protein other than soybean and allowed free access to water. They were gently managed; squeezing, pressure and tough maneuver were avoided. Anesthesia was used whenever applicable. In addition animals’ cadavers and parts of tissues were handled with care by following the principles of healthy hygiene, while dead bodies were incinerated in the National Research Center incineration.

Diet:

The modified diet consist of  protein 22% (concentrate animal protein, corn gluten meal), starch 67%, choline chloride 1%, sodium chloride 1%, vitamin and mineral mixture 3%, methionine 1%, D-lycine 1%, corn oil 3%, di-calcium phosphate 1% (Knapka et al., 1974).

Experimental groups:

Rats were allocated into 7 groups (n=6-8), sham operated rats received 10ml/kg of 1% tween 80 in distilled water, osteoporotic control rats received 10ml/kg of 1% tween 80 in distilled water. Osteoporotic rats received linseed oil (LO) at 2 dose levels (90, 180 mg/kg/day po), fish oil (FO) at 2 dose levels (90, 180 mg/kg/day po) and alendronate sodium (ALN) (3 mg/kg/day po).

All groups were kept on modified diet, ovariectomized groups were administered the tested drugs for 8 weeks then the anti-osteoporotic effect were evaluated through DEXA, serum osteocalcin, serum TRAP and serum TNF-α.

Drugs and chemicals:

Linseed oil was purchased (Medizen Pharmaceutical industries for Nabha, Egypt), fish oil was purchased (Solvay Pharmaceuticals, Kuwait) and alendronate sodium were purchased (Multi-Apex Pharma, Badr city, Egypt).

All tested drugs were freshly prepared by suspending them in 1% tween 80 in distilled water. All tested doses were given orally by gastric oral tube for 8 weeks.

Methods:

Rats were anesthetized with ketamine hydrochloride 50mg/kg and xylazine 10mg/kg to overcome the muscle rigidity (Green et al., 1981).

Bi-lateral ovariectomy was performed using a dorsal approach. Hair of both dorsal sides was shaved. A skin incision about (0.5-1cm long) was made midway between the last rib and the cervix and about 1cm lateral to the spinal muscles. A second parallel but shorter incision was made through the muscles under the incised skin area. The ovary embedded in fat was withdrawn out. A hemostat is clamped around the uterine vasculature between the oviduct and the uterus. Each ovary and part of the oviduct is removed with single cuts through the oviducts near the ovary and the end of the uterus returned back to the peritoneal cavity. The cut edges of the muscle were sewn with catgut and those of the skin with silk. The ovary of the other side was similarly removed through a separate incision. Penicillin G procaine (40,000U/kg) was injected intramuscularly to prevent bacterial infection. Sham-operated rats were subjected to the same surgical procedure except the ovaries were not removed (Frost et al., 1992).

The rats were fasted 12 h before blood sampling, which was carried out under light ether inhalation anesthesia. Blood samples were withdrawn from the retro-orbital venous plexus using a heparinized micropipette according to the method of Halperin after 4, 8 and 12 weeks of surgery to assure osteoporosis in ovariectomized groups and to determine zero time for treatment. Then samples were taken again at 4 and 8 weeks after treatment to compare the treated ovariectionized groups with the control and sham operated groups. Each blood sample was collected in test tube, then allowed to clot at room temperature and serum was separated by centrifugation at 4000 rpm for 15 minutes (Halperin, 1951) for estimation of serum osteocalcin, BALP, TRAP, and TNF-α.
Evaluation of bone density:

Sixty female rats were anesthetized with i.p. injection of ketamine hydrochloride (50mg/kg) and were lying in a prone position with posterior leg maintained in external rotation with tape. A DEXA scan by Norland XR-46 takes about ten minutes and is associated with minimal radiation exposure. During the procedure, the machine passes over the body and takes measurements of bone density by sending a thin, invisible beam of low-dose x-rays through the bones. This low energy x-ray measures the bone mineral content (BMC) and bone mineral density (BMD) of femur (Mylona et al., 2005).

Statistical analysis:

Data were expressed as mean ± S.E comparison between means were carried out using two-way ANOVA followed by Bonferroni as multiple comparison tests for all biochemical and DEXA analysis. A probability level less than 0.05 was accepted as being significant for all of tested parameters.

Results:

Effect of linseed oil, fish oil and alendronate sodium on the femur bone mineral density of ovariectomized osteoporotic female rats:

The femur BMD of osteoporotic rats after 12 weeks was declined by 1.5 folds from the sham operated group. The administration of LO (90, 180 mg/kg) showed time dependant increase in femur BMD by 28% and 39% after 4 weeks and by 54% and 75% after 8 weeks. Also, doses of FO (90, 180 mg/kg) significantly increased femur BMD by 37% and 41% after 4 weeks and by 59% and 73% after 8 weeks. ALN increased femur BMD by 39% respectively after 4 weeks and by 78% at after 8 weeks as compared to osteoporotic control. Fig. no. (1)

Effect of linseed oil, fish oil and alendronate sodium on the serum OCN of ovariectomized osteoporotic female rats:

After 12 weeks of ovariectomy in rats kept on modified diet, serum osteocalcin showed a pronounced elevation by 2 folds with respect to sham operated group. Compared to the osteoporotic control and basal value, Linseed oil (90, 180mg/kg) significantly mitigated the rise in serum OCN in a time dependant manner by 14% and 22% respectively after 4 weeks and by 31% and 42% at respective time interval. Fish oil (90, 180 mg/kg) decreased serum OCN by 28% and 32% after 4 weeks by 46% and 51% respectively after 8 weeks. ALN decreased serum OCN after 4weeks by 33% and by 49% after 8 weeks. Fig. no. (2)
Fig. 2: Effect of linseed oil, fish oil and alendronate sodium on serum osteocalcin of female ovariectomized osteoporotic rats at zero, one and two months of treatment. Each bar represents the mean ± S.E. of 6-8 rats for each group * (P<0.05) vs. sham operated, @ (P<0.05) vs. control at respective time, b (P<0.05) vs. zero time of the same group using Bonferroni as a post test.

Effect of linseed oil, fish oil and alendronate sodium on the serum TRAP of ovariectomized osteoporotic female rats:

Ovariectomy in rats kept on modified diet for 12 weeks resulted in marked elevation of serum TRAP that exceeded the normal sham operated value by 2 folds. Linseed oil (90, 180 mg/kg) with respect to the basal value and osteoporotic control decreased serum TRAP by 10% and 15% respectively after 4 weeks and by 29% and 38% after 8 weeks. FO (90, 180 mg/kg) serum TRAP showed time dependant decline by 20% and 25% after 4 weeks and by 49% and 48% after 8 weeks. Alendronate sodium (3 mg/kg) also markedly decreased serum TRAP after 4 weeks by 25% and by 43% after 8 weeks. Fig. no (3).

Fig. 3: Effect of linseed oil, fish oil and alendronate sodium on serum tartrate resistant acid phosphatase of female ovariectomized osteoporotic rats at zero, one and two months of treatment. Each bar represents the mean ± S.E. of 6-8 rats for each group * (P<0.05) vs. sham operated, @ (P<0.05) vs. control at respective time, b (P<0.05) vs. zero time of the same group using Bonferroni as a post test.
Effect of linseed oil, fish oil and alendronate sodium on the serum TNF-α of ovariectomized osteoporotic female rats:

Serum TNF-α showed significant increase in rats fed modified diet after 12 weeks of ovariectomy with respect to sham operated. LO (90, 180 mg/kg) revealed pronounced decrease in serum TNF-α after 4 weeks by 41% and 49% respectively. After 8 weeks the decline reached 68% and 79% respectively with respect to the basal and the osteoporotic control. FO (90, 180 mg/kg) showed significant decline in serum TNF-α by 43% and 52% respectively after 4, by 8 weeks the decline was 81% and 84% respectively. ALN (3 mg/kg) exhibited time dependant reduction of serum TNF-α by 55% after 4 weeks and by 84% after 8 weeks. Fig. no. (4).

Discussion:

Ovariectomized rat model is the most commonly used animal model of postmenopausal bone loss and has been reported to be an appropriate model to study cancellous bone changes in humans (Jee et al., 2001). Although osteoporosis occur naturally in humans, ovariectomy has been used as a method to induce estrogen deficiency which is similar to the postmenopausal women in that there is a rapid loss of trabecular bone mass and strength that occur shortly after ovariectomy (Giro et al., 2008).

Alendronate sodium has shown clear cut protection against vertebral, hip and any nonvertebral fractures. Similarly, stated that, increase in bone mineral density (BMD) is associated with greater decline in vertebral fractures in post menopausal women receiving alendronate therapy (Silverman et al., 2007). The role of alendronate in pathogenicity of osteoporosis could be explained as follows, alendronate has no direct effect on osteoblastic cells, it only restore the balance between bone formation and bone resorption by inducing apoptosis and impairing function of osteoclast thus mitigate the bone resorption (Reszka et al., 2004). Besides, it prevent apoptosis of osteocytes and osteoblastic cells (Stepan et al., 2003). Alendronate sodium markedly showed a prominent decreased in serum TRAP. this comes in agreement with (Garnero et al., 1994) who declares that treatment with alendronate inhibits increased bone resorption and thereby normalized the rate of bone turnover. The possible effect of alendronate on osteoclastic cells is due to inhibition of osteoclast adhesion to the mineralized matrix thus preventing osteoclast attachment to the bone surface through alteration in the cytoskeleton especially actin and in the ruffled border which is the characteristic membrane of the active osteoclast (Benford et al., 2001).

Likewise alendronate sodium significantly decreased serum TNF-α to reach values significantly lower than those found in the control ovariectomized. The present results comes in harmony with (Cantatore et al., 1999) who reported a marked decrease of serum TNF-α and IL-1 levels associated with improvement of abnormal bone turnover indices following alendronate administration. The effect of alendronate on TNF-α is probably due to inhibition of the monocyte/macrophage lineage in which osteoclast are included (Sansoni et al., 1995) resulted in reduction in the proliferation and functional activity of monocytes, also it suppress antigen
presentation by cells of monocyte/macrophage lineage and inhibit the production of bone resorbing TNF-α. IL-1 and IL-6 (Pennanen et al., 1995).

Regarding the present study, daily administration of linseed oil and fish oil antagonized the declining in bone mineral content by estrogen deficiency and notably normalized the bone mineral density (BMD) to its base line value. The current results was consistent with a previous study which stated that higher femur bone mineral density was observed with a low dietary ratio of n-6/n-3 PUFAs (Watkins et al., 2006). Also, two other studies revealed positive correlation between a low dietary ratio of n-6/n-3 PUFA s and bone formation markers osteocalcin, bone specific alkaline phosphatase and bone mineral density (Kruger et al., 1998).

Concerning the effect of PUFAs on bone formation markers; serum osteocalcin and serum BALP, a previous study reported that animals supplemented with EPA, DHA and LA/ALA (n-6/n-3 EFAs) combination further showed an increase in trabecular bone surface and increased tissue level bone formation rates (Watkins et al., 2000).

The effect of EPA, DHA in fish oil and the ratio of n-6/n-3 in linseed oil on bone formation may be due to increased serum level of EPA which resulted in decrease production of arachidonic acid (AA) from precursor metabolites thus reducing production of PGE\(_2\) via cyclooxygenaze and 5-lypoxygenaze system which when found in high concentrations enhances bone resorption. At low levels of PGE\(_2\), insulin-like growth factor is stimulated which is considered as a powerful master growth stimulator for bone, cartilage and muscles (Kruger et al., 2010).

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Concerning treatment with linseed oil and fish oil for 8 weeks in the present study, counteracted the effect of ovariectomy on rats and normalized serum TNF-α and serum TRAP. These results are consistent with a previous study that showed 2 months of treatment with linseed oil revealed significant decrease in serum TNF-α and IL-6. Therefore, reduction in osteoclastic activity and establishment of osteoblastic activity indicate down regulation of bone turn over in ovariectomized rats (Abdelkarem et al., 2011). A previous study stated that fish oil inhibited the pro-inflammatory cytokines in immune cells as well as kidney tissues from autoimmune disease animal model(Fernandes et al., 1996).

The antagonizing effect of fish oil and linseed oil on osteoclastic cells may be due to the effect of DHA which inhibited the production of PGE\(_2\) along with suppression of TNF-α more effectively than EPA (Li et al., 1999). It also down regulate COX-II activity in local tissues thus reduce RANKL expression on activated T-cells and inhibit nuclear factor kappa (NF-kB) activation in osteoclast progenitor thereby inhibit osteoclastogenesis (Schmitz et al., 2008).

Moreover, they enhance the activation of nitric oxide through EPA and DHA found in fish oil and linseed oil which is postulated to play an important role in bone metabolism (Das, 2002).

In conclusion, all treated osteoporotic groups had lower serum osteocalcin, serum TRAP and serum TNF-α with respect to the control ovariectomized group which resulted in increase of bone formation and decrease in bone resorption in tissue.

Fish oil is more effective than linseed oil in treatment osteoporosis and both can be used instead of alendronate sodium as they are natural products and there was no adverse effect has been stated for them.

Disclosures:

I am the corresponding author and I declare on behalf of all authors that conflict of interest for the current manuscript “Effect of linseed oil, fish oil and alendronate sodium on ovariectomy-induced osteoporosis in female rats.

References


