Original Article

Prevention of Osteopenia and Dyslipidemia in Rats after Ovariectomy with Combined Aspirin and Low-dose Diethylstilbestrol*

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Abstract

Objective To study whether effect of aspirin plus low-dose diethylstilbestrol is more effective and safer than high diethylstilbestrol dose alone on prevention of ovariectomy-induced osteopenia and dyslipidemia.

Methods Thirty-eight 4-month-old female SD rats were divided into baseline (BAS) group (*n*=6), sham operation group (*n*=8) and ovariectomy (OVX) group (*n*=24). The OVX group was further divided into vehicle treatment group (*n*=8), diethylstilbestrol (30 μg/kg·d) treatment group (OVX+D30 group, *n*=8), and aspirin (9 mg/kg·d) plus diethylstilbestrol (10 μg/kg·d) treatment group (OVX+A-D10 group, *n*=8). Their left tibiae were collected for the bone histomorphometric analysis in undecalcified sections. Left femurs were collected for the bone mineral density measurement.

Results The body weight and serum cholesterol were increased, while uterine weight and cancellous bone mass were decreased in OVX rats compared with the SHAM group. Cancellous bone mass was significantly increased, while body weight and bone resorption parameters were decreased in both A-D10 and D30 treatment group compared with OVX group. The rats treated with A-D10 showed significantly increased in bone formation parameters and decreased in serum triglyceride compared with the D30-treated rats.

Conclusion Aspirin plus low-dose diethylstilbestrol can effectively prevent osteopenia by reducing bone resorption, and is thus a better treatment modality for preventing dyslipidemia than high-dose diethylstilbestrol alone.

Key words: Aspirin; Diethylstilbestrol; Ovariectomy; Osteoporosis; Dyslipidemia; Rat

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INTRODUCTION

Osteoporosis is an emerging medical and socioeconomic threat characterized by a systemic impairment of bone mass, strength, and microarchitecture, which increases the tendency of fragility fracture. About 40% of white postmenopausal women are affected by osteoporosis, while about 88 million patients suffer from this disease in China, which is expected to

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steadily increase in the near future. Various fragility fractures represent major complications of the disease, vertebral or hip fractures and subsequent loss of mobility and autonomy lead to a great drop of the quality of life. Thus, prevention and treatment of osteoporosis should be aimed at substantially reducing the risk of fracture. Some agents have been suggested for treatment of postmenopausal osteoporosis, in addition to lifestyle modifications, vitamin D and calcium supplementation. For example, bisphosphonates are most widely used because they are effective and inexpensive. However, long-term use of bisphosphonates has some limitations and side effects, such as subtrochanteric femur fractures, osteonecrosis of the jaw, oesophageal irritation, *etc*, which cause reduction of patients' compliance. Therefore, it is necessary to choose effective medications which can not only reduce the risk of fracture but also are good for compliance of patients.

Estrogen replacement therapy (ERT), a primary therapy for prevention of osteoporosis in postmenopausal women, can alleviate the most common menopausal symptoms, prevent bone loss, reduce the risk of fracture, and improve the quality of life. Some observational studies suggested that ERT may be associated with a reduced risk of cardio and cerebral-vascular events. However, the incidence of breast cancer, endometrial cancer, thromboembolic disease, and ischemic stroke increases significantly due to use of ERT. Many women want to benefit from the hormone therapy, including protection against cardiovascular disease, osteoporosis and fracture, urogenital atrophy, skin atrophy and dementia. Thus, minimizing the risks of estrogen therapy for these women is an important consideration. It is encouraged to reduce the risk of side effects with a lower effective dose of estrogen which shows a beneficial effect on bone metabolism and vaginal tissue.

Aspirin is a widely used non-steroidal anti-inflammatory drug and plays a role in anti-thrombosis and prevention of cardiovascular events such as acute myocardial infarction, ischemic shock and peripheral vascular diseases. It is well known that the main undesirable side-effects of aspirin, including gastrointestinal ulcer, stomach bleeding, and tinnitus, occur especially with higher doses. Low doses of aspirin are considered to be safe in prevention of strokes and heart attacks. According to epidemiological studies, regular use of aspirin or non-steroidal anti-inflammatory drugs can improve bone mineral density (BMD) in postmenopausal women. However, it appears to have no clinical significance regarding the protective effect on the subsequent risk of fractures. It was also reported that continuous aspirin infusion shows a higher BMD level in OVX-induced osteopenic mice than in controls. Aspirin is also shown to be able to reduce osteoclasts activity in mice after OVX. When cultured marrow stromal cells (MSC) are treated with aspirin, they improve anti-apoptotic capacity and elevate mineralized tissue formation *in vitro* and *in vivo*. Our previous studies demonstrated that aspirin is potent in preventing cyclophosphamide-induced bone loss by promoting bone formation. It was recently reported that aspirin can significantly increase bone mass in rats after OVX. Thus, a small dose of aspirin may offer a new approach to the treatment of estrogen-deficient osteoporosis and dyslipidemia.

In the present study, a compound preparation (A-D10) containing aspirin (9 mg/kg·d) and diethylstilbestrol (10 μg/kg·d) was designed to study whether it has an additive effect on preventing OVX-induced osteopenia and dyslipidemia. It is aimed at reducing the side effects and risks of estrogen while making use of its benefits through the addition of aspirin.

MATERIALS AND METHODS

Reagents

Diethylstilbestrol (Hefei Jiulian Pharm Co., Ltd., Hefei, China, Lot: 091008), aspirin (Guangdong Nanguo Pharm Co., Ltd., Guangzhou, China, Lot: 091113), tetracycline (Shanghai New Asiatic Pharm Co., Ltd., Shanghai, China, Lot: 080423), sodium pentobarbitone (Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China, Lot: 061222), silver nitrate (Xilong Chemical Co., Ltd., Shantou, China, Lot: 090711), TWEEN-80 (Sinopharm Chemical Reagent Beijing Co., Ltd., Shanghai, China, Lot: 090912), methyl methacrylate (Beijing Chemical Factory, Beijing, China), dibutyl phthalate (Xilong Chemical Co., Ltd., Shantou, China, Lot: 100205), and benzoyl peroxide (Hubei University Chemical Factory, Hubei, China, Lot: 090305) were purchased as indicated. Calcein and toluidine blue were purchased from Sigma-Aldrich Co., MO, USA. Diethylstilbestrol and aspirin were dissolved successively in sterile saline (pH 7.2-7.4) by adding TWEEN-80 to prepare the A-D10 compound. Serum total cholesterol (TC, Lot: 20100105), triglyceride (TG, Lot: 20100112),

high-density lipoprotein cholesterol (HDL, Lot: 20091223), low-density lipoprotein cholesterol (LDL, Lot: 20100113), and alkaline phosphatase (ALP, Lot: 20100107) were measured with assay kits purchased from Nanjing Jiancheng Biological Bioengineering Co. Ltd (Nanjing, China). Instruments including ELX800 Microplate Reader (Bio-Tek Instruments, Inc., USA), low speed saw (Buehler Ltd., IL, USA), RM2155 hard tissue microtome (Leica AG, Wetzlar, Germany), image analysis system (Osteometrics, GA, USA), and dual-energy X-ray absorptiometry (DEXA) scanner (Norland Co., WI, USA) were used.

Animals and Treatments

Thirty-eight 4-month-old female SD rats (weighing 254±20 g) provided by the Animal Center of Guangdong Medical College, Zhanjiang, China were acclimatized to local vivarium conditions at temperature of 24-26 °C and a humidity of 70% with free access to water and a pelleted commercial natural diet containing 1.33% calcium and 0.95% phosphorus. After 10 days of adaptive feeding, the rats were divided into baseline (BAS) group (*n*=6), sham operation group (*n*=8) and ovariectomy (OVX) treatment (*n*=24). On Day 2 after operation, the sham operation group was treated with vehicle. The OVX rats which underwent bilateral OVX under general anesthesia in accordance with literature were further divided into vehicle treatment group (*n*=8) diethylstilbestrol (30 μg/kg·d) treatment group (OVX+D30 group, *n*=8), and aspirin (9 mg/kg·d) plus diethylstilbestrol (10 μg/kg·d) treatment group (OVX+A-D10 group, *n*=8). The drugs were given by oral gavage for 90 days. Body weight of the rats was recorded weekly. All rats were given subcutaneous injection with tetracycline (30 mg/kg) on days 14, 13 and calcein (10 mg/kg) on day 4, 3 before death. At the endpoint of experiment, rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and killed. Their blood was collected for biochemistry measurement, and their uteri were collected and weighed. Their left tibiae were collected for the bone histomorphometric analysis in undecalcified sections. Left femurs were collected for the bone mineral density measurement. The animals were treated in accordance with the Guide for Care and Use of Laboratory Animals by the National Committee of Science and Technology of China. The experimental protocol was approved by the Institutional Animal Use and Care Review Board of Guangdong Medical College, Zhanjiang, China.

Serum Markers Assay

Serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and alkaline phosphatase (ALP) were measured with assay kits.

Bone Histomorphometry

Left tibiae were removed and bone marrow cavities were exposed by using low speed saw (Buehler Ltd., USA). The proximal tibial metaphysis (PTM) was fixed in 10% buffered formalin for 24 h, followed by gradient alcohol dehydration, xylene defatting, and embedded undecalcification in methyl methacrylate. The frontal PTM tissue was cut into 9-μm and 5-μm thick sections with the RM2155 hard tissue microtome (Leica AG, Wetzlar, Germany). The unstained 9-μm sections were used for dynamic histomorphometric analysis. The 5-μm sections were stained with either silver nitrate or toluidine blue for static histomorphometric measurements. A semi-automatic digitizing image analysis system (OsteoMetrics, GA, USA) was used for quantitative bone histomorphometry measurements.

To avoid primary spongiosa, the measurement site on the bone sections was the PTM between 1 and 4 mm distal to the growth plate-epiphyseal junction. Static measurement parameters included the total area of bone tissue (T.Ar), trabecular area (Tb.Ar), trabecular perimeter (Tb.Pm), number of osteoclasts (N.Oc), and osteoclast surface perimeter (Oc.S). Dynamic measurement parameters included single-labeled perimeter (sL.Pm), double-labeled perimeter (dL.Pm), and interlabeled width (Ir.L.Wi). According to relevant formula, the following parameters were calculated, including percent of the trabecular bone area (%Tb.Ar), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), number of osteoclasts per milimeter (Oc.N), percent of osteoclast surface perimeter (%Oc.Pm), percent of labeled perimeter (%L.Pm), mineral apposition rate (MAR), bone formation rate per unit of bone volume (BFR/BV), bone formation rate per unit of bone surface (BFR/BS), and bone formation rate per unit of bone tissue area (BFR/TV). All histomorphometric parameters were in accordance with the published studies.

Bone Mineral Density (BMD) Measurement

The left femurs of rats were wrapped with

saline saturated gauze to maintain their moisture and stored at -20 °C. After thawed at room temperature, the femurs were moisturized by soaking them in saline solution with the residual muscle removed. The whole femur bone mineral density was scanned with A DEXA scanner (Norland Co. WI, USA) to measure the bone mineral content (BMC, g/cm) and bone width (BW, cm). The BMD was calculated as BMC/BW.

Statistical Analysis

Data were presented as mean±SD, and analyzed using SPSS12.0 software for Windows (SPSS Inc. 2003). Differences among groups were evaluated by ANOVA and Fisher's PLSD test. $P<0.05$ was considered significant.

RESULTS

Body Weight and Uterine Wet Weight

As shown in Figure 1, the body weight was significantly higher in vehicle treatment groups than in sham operation group ($P<0.05$) while the body weight was significantly lower in D30 and A-D10 treatment groups than in vehicle treatment groups two weeks after treatment ($P<0.05$, $P<0.01$), the body weight was 11.5% higher in vehicle treatment groups than in sham operation group and 16.2% and 10.9% lower in A-D10 or D30 treatment group at the end of study ($P<0.01$, $P<0.05$). As shown in Figure 2, the uterine wet weight was 78.8% lower in vehicle treatment groups than in sham operation group ($P<0.01$), and 272.2% and 233.3% higher in A-D10 or D30 treatment group than in OVX treatment group ($P<0.01$, $P<0.05$), no significant difference was found in body weight and uterine weight between A-D10 or D30 treatment group and sham operation group.

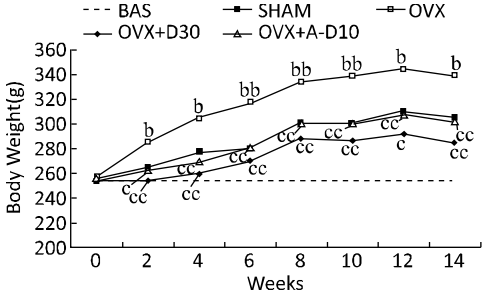

Figure 1. Body weights in different groups following 90 days of treatment. $^{b}P<0.05$, $^{bb}P<0.01$ *vs* sham operation group; $^{c}P<0.05$, $^{cc}P<0.01$ *vs* OVX treatment group.

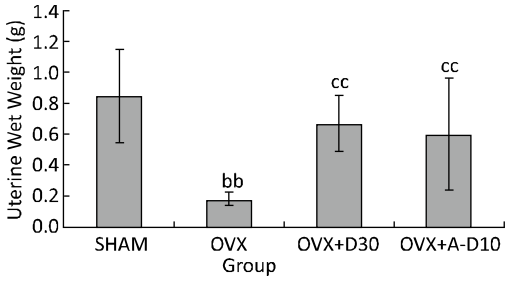

Figure 2. Uterine wet weights in different groups following 90 days of treatment. Data are expressed as mean±SD. $^{bb}P<0.01$ *vs* sham operation group; $^{cc}P<0.01$ *vs* OVX treatment group.

Serum Biochemical Markers

As illustrated in Figure 3, the serum levels of TC and ALP were 47.5% and 55.2% higher in vehicle treatment groups than in sham operation group ($P<0.01$, $P<0.05$) with no significant difference observed in serum levels of TG, LDL, and HDL among the above groups, while the serum level of TC and LDL was 59.8% and 23.6% ($P<0.01$, $P<0.05$) lower while that of TG was 30.3% higher in D30 treatment group than in vehicle treatment groups ($P<0.05$), the serum levels of TC, LDL, and TG were 52.7%, 44.1%, and 46.3% lower in A-D10 treatment group than in vehicle treatment groups ($P<0.01$, $P<0.05$, $P<0.01$) while the serum levels of LDL and TG were 26.8% and 58.7% lower in A-D10 treatment group than in D30 treatment group ($P<0.05$, $P<0.01$) with no significant difference found in HDL among the above groups.

Histomorphometry of Cancellous Bone

Static histomorphometric measurements of PTM were illustrated in Table 1, Figures 4 and 5. No significant change in static parameters (%Tb.Ar, Tb.Th, Tb.N, Tb.Sp, Oc.N, and %Oc.Pm) was found between BAS group and sham operation group. The bone mass (%Tb.Ar) was significantly lower in OVX treatment group than in sham operation group ($P<0.01$) which was accompanied with a significant decrease in Tb.Th and Tb.N, and a significant increase in Tb.Sp, Oc.N, and %Oc.Pm. %Tb.Ar was 124.2% and 108.2% higher in D30 and A-D10 treatment groups than in OVX treatment group ($P<0.01$), the Tb.N was 95.7% higher while the Tb.Sp, Oc.N, and %Oc.Pm were 64.5%, 39.6%, and 46.2% lower in A-D10 treatment group than in OVX treatment group ($P<0.01$). No significant difference was observed in static histomorphometric parameters between the two drugs treatment groups. However, OVX-induced bone loss could not be completely prevented in D30 nor A-D10 treatment groups compared with sham operation group.

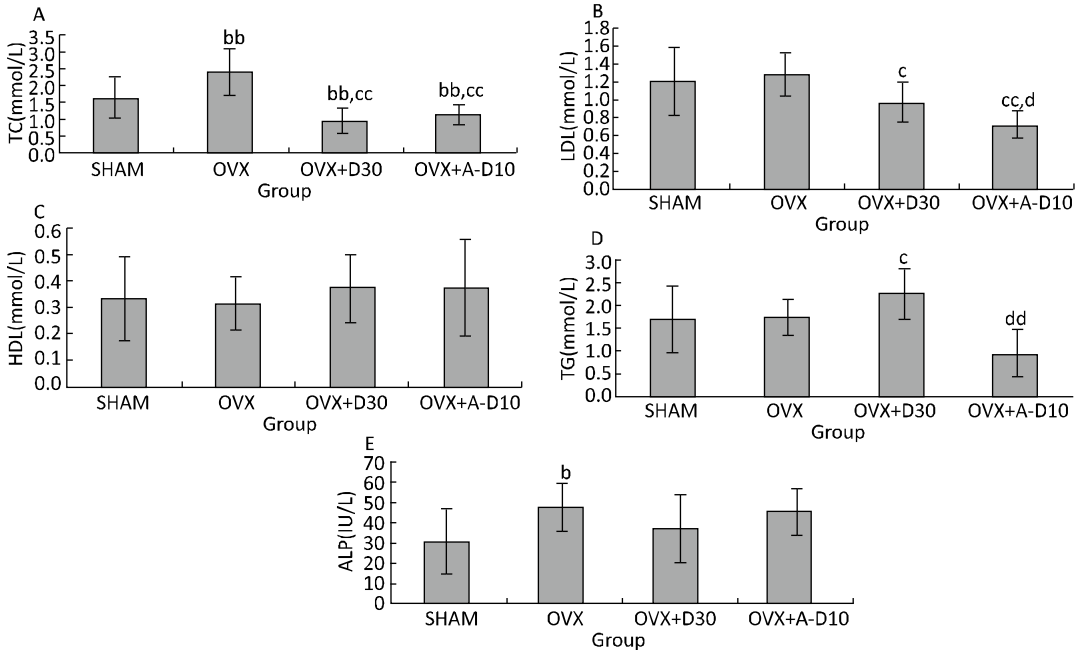

Figure 3. Serum levels of TC (A), LDL (B), HDL (C), TG (D), and ALP (E) in different groups. Data are expressed as mean±SD. $^{b}P<0.05$, $^{bb}P<0.01$ *vs* sham operation group; $^{c}P<0.05$, $^{cc}P<0.01$ *vs* OVX treatment group; $^{d}P<0.05$, $^{dd}P<0.01$ *vs* OVX+D30 treatment group.

Table 1. Static Histomorphometric Parameters of PTM Following 90 Days of Treatment (Mean±SD)

Group	%Tb.Ar (%)	Tb.Th (μm)	Tb.N (#/mm)	Tb.Sp (μm)
BAS	27.30±4.34	48.31±2.72	5.65±0.85	132.11±29.27
SHAM	29.70±4.73	51.91±4.74	5.70±0.55	125.02±20.16
OVX	8.90±4.27^{bb}	40.65±5.72^{bb}	2.12±0.87^{bb}	594.59±506.87^{bb}
OVX+D30	19.95±4.47^{bb,cc}	43.88±4.80^{bb}	4.51±0.62^{bb,cc}	181.45±32.96^{cc}
OVX+A-D10	18.53±4.71^{bb,cc}	44.53±4.12^{bb}	4.15±0.97^{bb,cc}	211.23±76.06^{cc}

Note. %Tb.Ar: percent of trabecular area; Tb.Th: trabecular thickness; Tb.N: trabecular number; Tb.Sp: trabecular separation. Data are expressed as mean±SD. $^{bb}P<0.01$ *vs* sham operation group; $^{cc}P<0.01$ *vs* OVX treatment group.

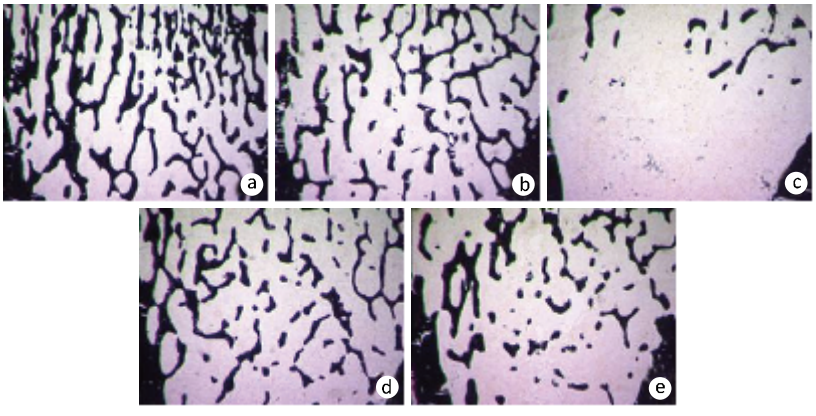

Figure 4. Proximal tibial metaphysis (PTM) in different groups. OVX-induced cancellous bone loss in PTM is prevented after D30 and A-D10 treatment. Silver nitrate staining, original magnification × 40.

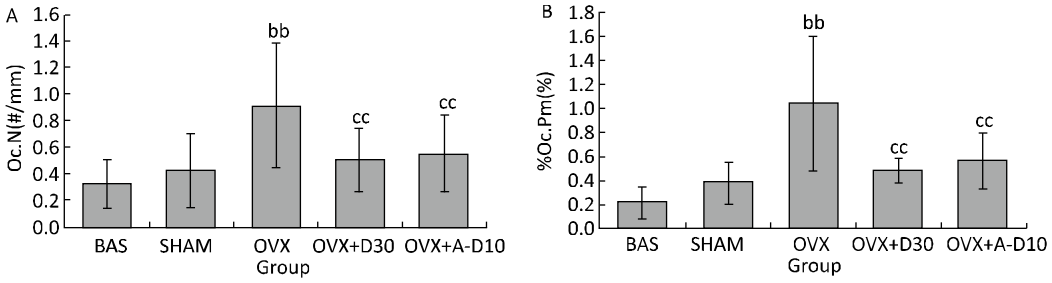

Figure 5. Osteoclasts per millimeter (Oc.N, A) and percent of osteoclast surface perimeter (%Oc.Pm, B) of PTM in different groups. Data are expressed as mean±SD. $^{bb}P<0.01$ *vs* sham operation group; $^{cc}P<0.01$ *vs* OVX treatment group.

Dynamic histomorphometric measurements of PTM are illustrated in Table 2 and Figure 6. No significant change in dynamic parameters (%L.Pm, MAR, BFR/BS, BFR/BV, and BFR/TV) was found between BAS group and sham operation group. The bone formation (%L.Pm) and BFR/BV were 33.5% and 51.0% higher in OVX treatment group than in sham operation group ($P<0.05$). MAR, BFR/BS, and BFR/TV did not change significantly in OVX treatment group. The %L.Pm was 29.2% lower in D30 treatment group than in vehicle treatment groups ($P<0.01$). However, no significant difference was observed in bone formation parameters between vehicle treatment groups and A-D10 treatment groups. In addition, the %L.Pm was higher in A-D10 treatment group than in D30 treatment group ($P<0.05$) with no significant difference found in MAR, BFR/BS, BFR/BV, and BFR/TV between the two groups.

Table 2. Dynamic Histomorphometric Parameters of PTM Following 90 Days of Treatment (Mean±SD)

Group	%L.Pm (%)	MAR (μm/d)	BFR/BS (%/yr)	BFR/BV (%/yr)	BFR/TV (%/yr)
BAS	18.3±1.2	0.91±0.18	16.8±3.3	211.0±37.6	57.2±10.9
SHAM	18.2±2.8	0.94±0.11	17.0±3.4	200.3±38.9	59.2±13.9
OVX	24.3±6.8^{b}	0.98±0.18	20.5±8.3	302.4±105.6^{b}	61.8±17.6
OVX+D30	17.2±5.1^{cc}	0.84±0.15	17.2±7.8	242.2±101.7	46.8±18.7
OVX+A-D10	22.5±3.9^{d}	0.85±0.12	19.2±4.9	265.7±72.0	48.7±18.9

Note. %L.Pm: percent of labeled perimeter; MAR: mineral apposition rate; BFR/BS: bone formation rate/bone surface; BFR/BV: bone formation rate/bone volume; BFR/TV: bone formation rate/tissue volume. $^{b}P<0.05$, $^{bb}P<0.01$ *vs* sham operation group; $^{c}P<0.05$, $^{cc}P<0.01$ *vs* OVX treatment group; $^{d}P<0.05$, $^{dd}P<0.01$ *vs* OVX+D30 treatment group.

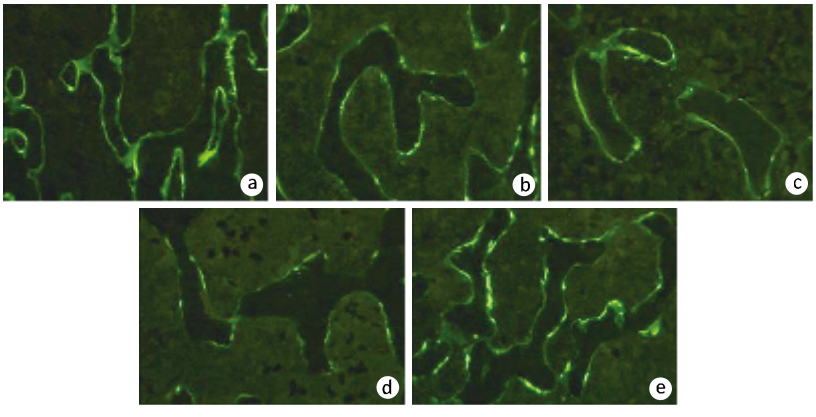

Figure 6. Fluorescence showing trabeculae of proximal tibial metaphysis (PTM) after different treatments (original magnification×100).

Bone Mineral Density (BMD)

As shown in Figure 7, The BMD was significantly lower in vehicle treatment groups than in sham operation group (P<0.01), and was 26% and 26% higher in D30 and A-D10 treatment groups than in vehicle treatment groups (P<0.01) with no significant difference in BMD between D30 and A-D10 treatment groups.

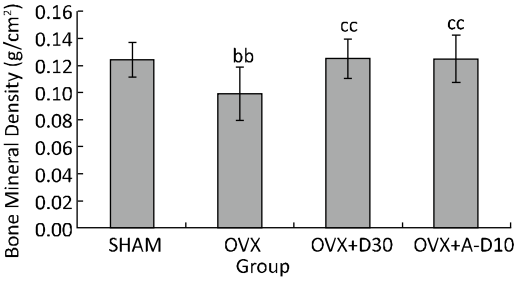

Figure 7. BMD of the left femurs in different groups. Data are expressed as mean±SD. ^{bb}P<0.01 *vs* sham operation group, ^{cc}P<0.01 *vs* OVX treatment group.

DISCUSSION

This study demonstrated that low-dose diethylstilbestrol and aspirin compound preparation (A-D10) prevented bone loss and decreased blood lipid markers induced by OVX, and the effect of A-D10 on preventing bone loss was similar to that of higher-dose diethylstilbestrol (D30). Interestingly, A-D10 decreased blood lipid markers while D30 increased serum triglyceride level. According to the conversion of drug dosage between rats and humans, the dosage of aspirin (9 mg/kg·d) in the A-D10 preparation is similar to that (100 mg/d) for preventing humans cardiovascular events. The dosage of diethylstilbestrol (30 μg/kg·d, D30) is similar to that for preventing humans cardiovascular events, which is considered to be a routine dose in ERT, while the low diethylstilbestrol dose (10 μg/kg·d, D10) in A-D10 was only one third of the routine dose. In this study, we have not designed a (OVX+D10) group and a (OVX+A) group. Our previous study investigated the effects of 17α-ethynylestrogen (100 μg/kg·d and 30 μg/kg·d) on rats after OVX, which were similar to 30 μg/kg·d and 10 μg/kg·d of diethylstilbestrol. The results showed that 17α-ethynylestrogen at 100 μg/kg·d markedly increased bone mass by 205%, while 17α-ethynylestrogen at 30 μg/kg·d only partially increased bone mass by 105% and that diethylstilbestrol at 30 μg/kg·d significantly increases the BMD of femurs in rats after OVX, that diethylstilbestrol at 22.5 μg/kg·d significantly prevents trabecular bone loss by reducing high bone turnover, which inhibits both bone formation and resorption. It was reported that ERT prevents osteoporosis in a dose-dependent manner. Our previous studies demonstrated that a low aspirin dose (9 mg/kg·d) is potent in preventing cyclophosphamide-induced cortical and trabecular bone loss by promoting bone formation. It was recently reported that aspirin at 8.93 mg/kg·d (≈9.0 mg/kg·d in this study) significantly increases BMD of lumbar vertebrae 2-6 and significantly increases trabecular thickness and trabecular number. Therefore, in this study, rats after OVX were treated with diethylstilbestrol at 30 μg/kg·d, and one-third of the diethylstilbestrol dose plus aspirin (9 mg/kg·d) was chosen to produce the A-D10 compound preparation.

Bone histomorphometry in this study demonstrated that the trabecular bone of the rats after OVX showed typical osteopenia and high bone turnover after 90 days of treatment. The bone resorption and formation parameters were significantly lower in rats after treated with D30 than after treated with OVX, suggesting that ERT can suppress OVX-induced high bone turnover. In this study, trabecular bone mass, BMD of femurs and uterine wet weight were significantly higher in rats after D30 treatment than in those after OVX treatment, suggesting that ERT can effectively prevent OVX-induced osteopenia. However, the bone mass did not reach its value in sham operation group in this study, which is consistent with that reported in other studies.

The serum TC, LDL and cholesterol levels were significantly lower while serum TG level was significantly higher in D30 treatment group than in vehicle treatment groups. The uterine wet weight was significantly higher in D30 treatment group than in OVX treatment group, suggesting that diethylstilbestrol at 30 μg/kg·d increases TG level and endometrium proliferation, which is the risk factors for thromboembolic diseases, ischemic stroke, and endometrial cancers in humans. However, the beneficial role of estrogen in prevention of fracture, protection from cardiovascular diseases, urogenital atrophy, skin atrophy and dementia is worthy of recognition. A recent study suggested that using the lowest effective dose of estrogen can decrease the

side effects and bleeding while the low-dose therapy is associated with beneficial effects on bone metabolism. At present, no report is available on what is the minimum effective dose of ERT in large randomized clinical trials. However, a lower dose of ERT can partially prevent postmenopausal bone loss and appears to be a useful alternative to higher dosages in prevention and treatment of climacteric symptoms in postmenopausal women with fewer side effects and lower risks. Thus, lower-dose estrogen replacement therapies for osteoporosis prevention should be encouraged.

The data in this study demonstrate that after 90 days of A-D10 treatment, the bone loss was significantly higher in vehicle treatment groups than in D30 treatment group. In addition, A-D10 significantly reduced bone resorption parameters such as Oc.N and %Oc.Pm with no effect on bone formation parameters. The %L.Pm was 30.8% higher in A-D10 treatment group than in D30 treatment group ($P<0.05$), indicating that A-D10 treatment is different from D30 treatment. A-D10 inhibited bone resorption but not bone formation showing that combined aspirin and low diethylstilbestrol dose can reduce osteoclast activity and maintain osteoblast activity in rats after OVX treatment.

The role of estrogen in prevention of ovariectomy-induced osteopenia has been fully demonstrated. The effects of estrogen on bone metabolism are mediated by the binding of hormone to a specific receptor. The estrogen-estrogen receptor complex then functions as a gene specific transcription factor, subsequently regulating osteoblast differentiation and activity and inducing apoptosis of bone-resorbing osteoclasts. However, the mechanism of aspirin underlying prevention of bone loss in rats after OVX is still unclear. It was reported that aspirin can inhibit COX-2 function and regulate PGE_2 expression, thereby inhibiting the osteoclast function and preventing osteoporosis. It has been shown that aspirin can prevent apoptosis of bone marrow stromal cells (MSC) *in vitro*, reduce the colony stimulating factor-F (CFU-F), promote differentiation of MSC into osteoblast, and inhibit osteoclast function. Some reports demonstrated that aspirin can prevent OVX-induced osteopenia by inhibiting T-cell activation, thereby inhibiting the Fas/FasL pathway-mediated apoptosis of MSC and osteoclast-related bone resorption. The present study showed that combined aspirin and low diethylstilbestrol dose could inhibit osteoclast activity but not its activity by inhibiting osteoclast activity, stimulating differentiation of MSC into osteoblast, preventing MSC from apoptosis, regulating blood lipid metabolism, and promoting blood circulation in bone marrow. Further research is needed to determine whether aspirin and low diethylstilbestrol dose have synergistic effects.

This study further demonstrated that the serum TC and LDL levels were significantly lower in A-D10 treatment group than in OVX treatment, suggesting that A-D10 is effective in preventing ovariectomy-induced dyslipidemia. The serum TG and LDL levels were significantly lower in A-D10 treatment group than in D30 treatment group, suggesting that A-D10 can prevent hypertriglyceridemia and is beneficial to lipid metabolism in rats after OVX. Hypertriglyceridemia is an important risk factor for cardiovascular diseases, and is strongly associated with increased inflammation. Therefore, inhibition of inflammation is a potential therapeutic target of hypertriglyceridemia in rats after OVX. Interestingly, aspirin can diminish hypertriglyceridemia in obese rodents and patients with type 2 diabetes mellitus. Recent data indicate that aspirin inhibits NF-κB and decreases hypertriglyceridemia by reducing hepatic (LDL-TG) secretion rather than by accelerating its distribution in hepatic tissue.

Moreover, the uterine weight is lower in A-D10 treatment than in D30 treatment group, indicating that A-D10 cannot stimulate uterus overgrow as low estrogen dose. Although no study is available on relation between use of aspirin and endometrial adenocarcinoma, data from a prospective cohort study suggest that while aspirin does not play an important role in endometrial cancer risks, risks are significantly lower in current aspirin users who are obese or postmenopausal and have never used postmenopausal hormones. Furthermore, aspirin inhibits COX and thereby reducing prostaglandin synthesis. It was reported that abnormal up-regulation of COX and prostaglandins is a feature of breast cancer, suggesting that aspirin may have potential value in its treatment and prevention.

In conclusion, combined aspirin and low-dose diethylstilbestrol can effectively prevent bone loss by reducing bone resorption and preventing dyslipidemia in rats. It indicated that A-D10 have the potential of preventing osteoporosis, normalizing blood lipids in postmenopausal women. Further study is needed to optimize the dosage and make clear the interaction between aspirin and diethylstilbestrol.

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