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Original Article

Effect of repaglinid on ovariectomy induced bone loss in rats: comparison with 17β-estradiol

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Abstract

Aim: The aim of this study was to investigate the effect of repaglinide (RPG), anti-diabetic drug used commonly diabetic patients, on bone loss ovariectomized (OVX) rats".

Material and Methods: Forty Wistar albino rats were equally divided into five groups as follows: Group 1, control, non-OVX rats; Group 2, OVX rats administered 0.1 % ethanol; Group 3, OVX rats administered subcutan 17β-estradiol (E2) at a dose of 40 µg/kg; Group 4, OVX rats administered oral RPG at a dose of 0.5 mg/kg; Group 5, OVX rats administered E2 (40 µg/kg) and RPG (0.5 mg/kg) rats. Administration of drugs started 5 days after bilateral ovariectomy and continued for 35 days. After 35 days of treatment, the animals were sacrificed and the whole femurs were dissected and were analyzed for bone mineral density (BMD) and bone mineral content (BMC) by dual-energy x-ray absorptiometry (DXA).

Results: There were no significant difference between OVX and RPG groups for BMC and BMD values. Compared with the OVX groups, E2 administration of OVX groups had higher BMC but no significant difference for BMD. OVX group had significantly lower value than the control group.

Conclusion: RPG administrated to OVX rats for 35 days had no beneficial effect on bone loss status in OVX rats.

Keywords: 17β-Estradiol; Repaglinide; Ovariectomy; Bone Mineral Content; Bone Mineral Density.

INTRODUCTION

Osteoporosis, which places an important economic burden on whole societies, families and health systems is a serious public health concern with as a growing worldwide incidence (1). Osteoporosis is characterized by a reduction and deterioration of bone tissue, consequently increasing bone fragility and susceptibility to fracture (2). Women are more prone to bone-loss than elderly men following postmenopausal estrogen deficiency. OVX animals are used to examine the mechanisms responsible for menopause-related complications in humans (3). Recent studies have reported that OVX rats experience an increased frequency of diabetes, cardiovascular complications and osteoporosis compared with normal rats (4). Thus, alternative or other therapeutic strategy with a proven efficacy and safety should be developed for the prevention and treatment post menopause-induced bone loss.

The estrogen shortage associated with the menopause is found to be the main cause of the changes in bone reconstruct that lead to the disorder known as osteoporosis (5). Most of the changes can be reduced and eliminated with estrogen replacement therapy (ERT). Against its benefits, treatment with estrogens have been indicated to cause serious negative effects including stroke and gallbladder disease, as well as endometrial, uterine and breast cancers (6). Recent researches have been directed towards the search for substitutes for estrogen treatment that may play role in a similar way on the bone metabolism without the adverse effects (7). Thus, there is increasing interest among patients in other alternatives to conventional hormonal therapy.

Repaglinide (RPG), S(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)- butyl) amino)-2-oxoethyl) benzoic acid, is chemically unrelated to the oral sulfonylurea insulin secretagogues. RPG has a large utility as a therapeutic agent in the treatment of diabetic patients (8). Recent research shows that RPG may play a role for fracture for older diabetic patients (9). RPG was a kind of non-sulfonylureas that was prescribed for unsteady postprandial glucose regulation in order to prevent hypoglycemia (8). However, the effects of RPG on bone metabolism have not fully been understood.

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There are no available publications on the influence of that drug on the development of osteoporosis in postmenopausal women; therefore, the aim of this study was to examine the effects of both E2 and/or RPG (0.5 mg/kg body weight) administered for 35 days on the development of osteopenia induced by bilateral ovariectomy in rats.

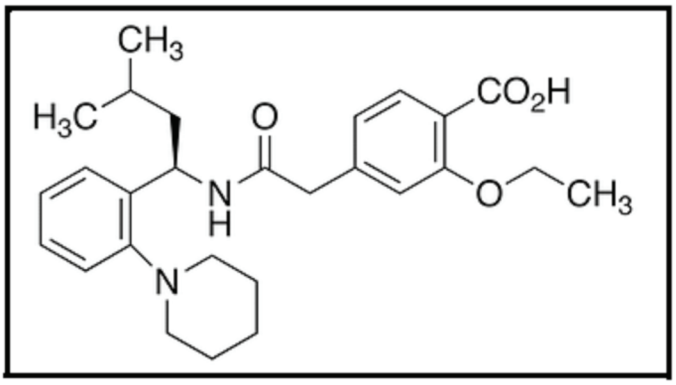

Figure 1. Structure of repaglinid

MATERIALS and METHODS

Forty female, adult, albino Wistar rats weighing 250 ± 20 gm (mean ± SD) at 3 months of age, were studied. They were housed three per cage at a temperature of 22±2°C with 12 h light/dark cycle under a controlled environment. The animals were fed on standard laboratory rodent chow and permitted drink water ad libitum. The permission for the animal tests and experiments was approved by the Elazig Veterinary Control Institute Local Ethics Committee for Animal Experiments. The rats, except for in the control group were bilaterally ovariectomized under intraperitoneal ketamine (70 mg/kg) and xylazine (10mg/kg) anesthesia, using dorsal approach. Rats in the control group (n=8) underwent a surgical procedure similar to the other groups but the ovaries were not removed. The OVX group rats (n=32) were randomly divided into four groups: OVX group (Sham, n=8); OVX+E2 group (n=8); OVX+RPG group (n=8); OVX+ E2+RPG group (n=8). The rats E2 treatment groups received 17β-estradiol (Sigma, Saint Louis, MO) dissolved in small amounts of ethanol with the volume adjusted with olive oil to give a concentration of 40 µg/kg body weight and were administered daily subcutaneously. The rats in RPG groups were received repaglinide (Sigma, Saint Louis, MO) dissolved in distilled water to give of a concentration 0,5 mg/kg body weight and were administered intragastrically using a feding tube. Each morning all the animals were weighed immediately before administration of the tested preparations.

That enabled us to determine an increase in rat's body weight and administer a proper E2 and RPG dose, with respect to body weight. Administration of E2 and RPG started on days 4 post-OVX and continued for 35 days. One day before the animals were euthanized, each rat was housed individually without food for 24 h in a metabolic cage. On the following day, sacrifice was performed when the rat was anesthetized with an intraperitoneal injection with 3% sodium pentobarbital (50mg/kg). Femurs were dissected, identified and stored in normal saline at -20˚C for bone analyses. According to the previous description (10), BMC and BMD of each rat's right and left femurs were measured by dual-energy X-ray absorptiometry (DXA). BMD was calculated using the BMC of the measured area and reported as g/cm2.

Statistical analysis: A comparison of the results of the different groups was performed with use of the Anova analysis of variance and post-hoc multiple comparison test. The results are given in the text as means ± STD. For all comparisons, statistical significance was defined as $p < 0.05$.

RESULTS

Our study examined the effects of RPG on BMD and BMC in a rat osteoporosis model which was designed by ovariectomy. Forty days after ovariectomy, BMD and BMC values of the OVX rats became significantly lower than the control group. This shows that our rat osteoporosis model is beneficial to evaluate the effect of E2 and RPG on BMD and BMC. Figure 1 shows femoral BMD and BMC values for the different groups. Total femur region, BMD and BMC values were expressed as the mean of the values obtained for femur. There was no significant difference between OVX and RPG groups for BMD and BMC measurements. Compared with the OVX group, OVX+E2 group had significantly higher values for BMC values, but were no significant for BMD values. Compared with the control group, OVX group had significantly lower values fir BMD and BMC values. The difference between RPG and E2 groups were not significant figure 1. BMD and BMC values of total femur region of rats, all groups.

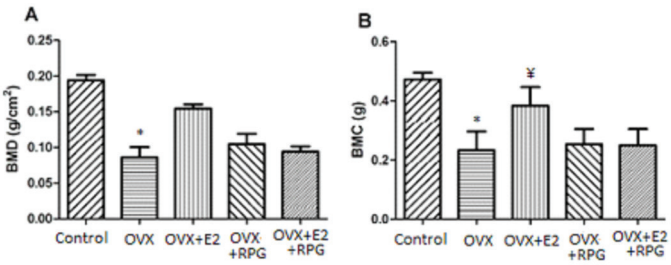

Figure 2. Changes in femoral BMD and BMC (measured by DEXA) treated RPG alone and with the addition of 17β-estradiol (E2) for 35 days, (mean ± SD, n=8). *p<0.05 as compared with group Control. ¥p<0.05 as compared with group OVX.
*The mean difference is significant at the 0.05 level

DISCUSSION

In the skeletal architecture of an adult, the bone remodeling and modelling process in which new bone constituents replace the old are repeated in throughout life, and the new and old bones continue a condition of balance. However, after menopause, both the accelerates of bone resorption increase and bone formation

(osteogenesis) decrease (11). For a disease such as osteoporosis, the rate of osteogenesis decreases despite bone resorption increases. In this study, bone resorption markers were higher in OVX rats in comparison to the control group, indicating a decrease in BMD and BMC. This correlated with low BMD and BMC values, which was also observed in a study by others (12,13). On the other hand, no significant difference was monitored in terms of osteogenesis markers and of the RPG supplementation group in comparison to the OVX group. However, bone resorption markers were significantly higher and indicate that BMC was higher in only E2 administration group in comparison to the OVX group. A recent study has reported the OVX rat to be a proper preclinical model for postmenopausal osteoporosis that truly reproduces the changes determined in humans and has the added benefit that the effects are detectable only a few months after operation (14). Celik et al. (15) showed that estrogen deficiency occurring 30 days after bilateral ovariectomy in sexually adult female rats also causes changes in the bone metabolism associated with loss bone mass. In agreement with the results of previous experiments (3,4) bilateral ovariectomy in our rats was proved to be a good experimental type for postmenopausal osteoporosis.

The role of estrogen on bone metabolism has been proved through estrogen replacement treatment. One study suggests that estrogen treatment prevent bone loss by regulating the production of cytokines that modulate osteoclastic bone resorption, involving interleukins (IL-1, IL-6, IL-11) and osteoprotegerin by cells of the osteoblastic (16). Estrogens also regulate the rate of cell-intrinsic suicide program of mature bone cells, creating pro-apoptotic effects on osteoclasts cells and antiapoptotic effects on osteoblasts cells (17). Our study shows that 35 days after E2 treatment there is a significant increase in femoral bone mineral content of OVX+E2 rats compared with values of BMC monitored in OVX groups. This reduction in BMC has also been identified by another study (18).

In this study, OVX 3-week-old Wistar–Albino rats were raised for 40 days. They were given RPG (0.5 mg/kg body weight/day) orally to study the role of short-term RPG supplementation on excremental osteoporosis. Supplemental RPG given to the OVX group did not prevent the effects of ovariectomy associated with decreases in the levels of bone mass and treated rats showed no significant differences from OVX rats. Similar results were also observed in the study of Vierte et al. (19) The effects of RPG on long bone development seen in all studies only occurred at excessively high plasma concentrations that will not be reached at human therapeutic doses. However, a recent study have showed that using RPG can increase the risk of fracture, through various mechanisms, in type 2 diabetic patients (between 65 t0 74 years of age) (9).

CONCLUSION

The results of current study suggest that the RPG does not attenuate the reduction in BMD and BMC generally observed after ovariectomy. E2 also prevents the loss of bone mass attributed to ovariectomy.

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