Research Article

Overiectedomized model and femur evaluation parameters for screening of antiosteoporotic Activity

S M Gunjegonkar1,*, S S Shulka1, A A Shirsath1 and S B Wankhede2

1Department of Pharmacology, JSPM’s Charak College of Pharmacy and Research Wagholi, Pune, Maharashtra
2Department of Pharmaceutical Chemistry, JSPM’s Charak College of Pharmacy and Research Wagholi, Pune, Maharashtra

*Corresponding Author: gunjeshiv@gmail.com

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ABSTRACT

The objective of the present study was to perform the ovariectomy in experimental animals and to study femur evaluation parameter for antiosteoporotic activity. Ovariectomy or Sham operations were performed on Wistar albino rats under the ketamine anesthesia. Ovariectomy means removal of ovaries by surgical procedure. Changes in femur physical parameters like Femur wet weight, Femur length, Femur volume and Femur density due to the removal of the ovary in the rat were studied. Ovariectomy was successfully performed in albino wistar rats and femur evaluation parameters were studied. Ovariectomized model is a promising model to induce the osteoporosis condition similar to postmenopausal women and major bones are getting affected due to lack of estrogen.

Introduction

Osteoporosis is an area of health interest since it affects the old age people and particularly the postmenopausal women because of estrogen deficiency after cessation of menopause (Frost 1992). The etiology of human osteoporosis is multi-factorial, including heredity, hormonal excess or deficiency, dietary components, and physical activity (Riggs 1986). Osteoporosis is a systemic skeletal disease, chronic and progressive condition and associated with low bone mass and deteriotiation of bone tissue at micro levels. Characteristic sites of fracture include vertebral bodies, distal radius, and proximal femur but the osteoporotic individuals have generalized skeletal fragility (Peak 1993; Shirwaikar 2003). The main reason behind postmenopausal osteoporosis is depletion of the hormone estrogen.

Postmenopausal osteoporosis is characterized by an increase in bone resorption relative to bone formation, and so progressive decrease in bone mass leads to an increased susceptibility to fractures, which result in morbidity and mortality. Vertebral fractures are important because they can cause pain, kyphosis, height loss and independently of bone mineral density (Burger 1994). Estrogen replacement therapy has been established a regime for prevention of postmenopausal bone loss and can prevent the early phase of involution bone loss.
and reduces fracture risk by about 30%. Thus, it is clear that the menopausal bone loss is attributable to the cessation of ovarian function and tapering-off of estrogen secretion (Torgerson 2001; Stevenson 2005).

Materials and methods

Induction of Osteoporosis in Rats

The acclimatized rats were either sham-operated or ovariectomized using the ventral midline skin incision. The rats underwent the surgical procedure after being anaesthetized with Ketamine (70mg/kg, i.m) (Yongzhong 2006; Zhen-Guo 2009). The rat was put on its dorsal surface and ovariectomy was preceded by a single longitudinal skin incision on the ventral midline of the abdomen, 2 cm long. Peritoneal cavity was opened and fat tissues were removed. Ligation of blood vessels was necessary. Both ovaries were identified and then silk thread was tightly tied around the oviduct, including the ovarian blood vessels. The oviduct was cut and the ovary was removed, taking good care in leaving the knot intact. The muscle incision was required suturing with 0 size chromic absorbable catguts (Junko 2004). The skin was sutured with non-absorbable silk thread. The rats of the sham group underwent sham surgery. Sham surgeries received the same incisions as the ovariectomized animals and were sutured in the same way, but the ovaries were palpated instead of removed. Broad spectrum antibiotic Neosporin antibiotic powder was used topically after surgery, once a day, for 10 consecutive days. The ovariectomy was confirmed by prominent atrophy of the uterine horns (Yan Zhang 2007; Gass 2001).

Experimental details

Body weights of all animals were recorded at the beginning and at weekly intervals throughout the experiment. At the end after overnight fasting, all the animals were weighed and blood samples were collected by puncturing the retro-orbital plexus under mild ether anesthesia. After clotting, blood samples were centrifuged to separate serum. Serum was collected and assessed for biochemical parameters. Once blood was collected, animals were sacrificed by cervical dislocation. The right and left femurs and right tibias were removed. The right femurs were cleared from soft tissue used for femur physical parameters and ash analysis. The left femurs were fixed in 10% neutral buffered formalin for histopathological examination. The right tibias were wrapped in gauze saturated with physiological saline and frozen till biomechanical analysis (Donahue 1988; Rao 1974).

Evaluation parameters

1) Femur physical parameters
2) Femur ash weight and calcium content
3) X-RAY image of the femur bone
4) Femur histopathology

1. Femur physical parameters

The right femurs, which was cleaned from surrounding tissue and weighed using Sartorius electronic weighing balance. This was the wet weight of femur. Femur length was measured by using Mitutoyo digital caliper (Made in China). Femur length is defined as the distance between the greater trochanter and medial condyle or distance from the proximal tip of the femur head to the distal tip of the medial condyle. Femur diameter was measured at midshaft femur by using Mitutoyo digital caliper. After measuring above parameter, bones were defatted in acetone for 2 days. Then defatted femur was allowed to dry in an oven at temperature 110ºC for 12 hr for several days till they attain a constant weight (Donahue HJ 1988; Stevensson J 2000)The dry weights of bones were determined by using Sartorius electronic weighing balance. Femur bone volume and density were measured by Archimedes’ principle. In osteoporosis, bone mineral density decrease and so the density of bone will be less. Here, we find bone density by using Archimedes’ principle. This principle will permit you to find the density of several solid materials and of a liquid sample.
a) Archimedes Principle
The principle of Archimedes is called as the principle of buoyancy or, simply, Archimedes principle is a body immersed in a fluid is buoyed up by a force equal to the weight of the displaced fluid (Donahue 1988; Archimedes Principle 2004). The principle applies to both floating and submerged bodies and to all fluids i.e., to liquids and gases and to solid materials that behave plastically over long spans of geologic time. Whether a given body will float, sink, or remain static in a given fluid is dependent on both the weight and volume of that material. The relative density, the weight per unit volume of the body contrasted to that of the fluid determines the buoyant force. If the body is less dense than the fluid, it will float, or, in the case of a balloon, it will rise. If the body is denser than fluid, it will sink. If the object has the same relative density, it will remain static i.e., it will neither rise nor fall (Islam 1998).

b) Buoyant Force
When an object is immersed in a fluid as in Fig 1, the fluid pressure acts perpendicular to the surface of the object at every point of its surface. Pressures acting on one side of the object cancel pressures acting on the opposite side. However, the pressure acting on the bottom of the object is always greater than the pressure acting on the top of the object. This causes a net upward force on the object, which is called the buoyant force or, simply, the buoyancy.

In Figure 2 (a), the spring scale just balances the force of gravity on the object, and thus the scale reads the weight of the object. In Figure (b), the buoyant force acts upward on the object, and the scale reads less than in Figure (a) by an amount equal to the buoyant force. We call this second reading the apparent weight of the object. The buoyant force is calculated as Buoyant Force = Weight – Apparent Weight.

Density: - The density ($\rho$) of a material of mass ($m$) and volume ($V$) is given by the equation: $\rho = \frac{m}{V}$

c) Procedure for finding femur bone volume and density
The weights of dried bones in the air and in water were determined. The right femur bones were dried and weight was taken by using Sartorius electronic weighing balance. This was called weight of bone in the air. Later, beaker of 250 ml filled with deionized water was taken. The bones were reweighed in completely immersed and suspended in water previously equilibrated to room temperature. This was called weight of bone in water. The equipment setup was used for performing the experiment as shown in the figure. Bone volume and density were then calculated according to the below formula (Archimedes Principle 2004).

Volume (ml) = mass in the air – mass in water
Density of water: Density (g/ml) = mass in air/volume

2. Femur Ash Weight and Calcium Content
For ashing, the right dried femurs of rats were placed in porcelain crucible that can withstand high temperatures and weighed. These crucibles were then placed in a muffle furnace and heated at 7500 C for 8 hr. After they had attained the room temperature the crucibles were weighed again. The ash weight was determined by using Sartorius electronic weighing balance. Femur ash content for calcium was estimated by below procedure. Ash was dissolved in 1ml of 6M Hydrochloric acid and then made up to 10 ml with deionized water and assayed for calcium content using autoanalyzer technique (Rao 1974; Islam 1988).

3. Femur X-RAY
X-ray technique is also a promising way to evaluate the changes in bone architecture and internal changes.

4. Femur Histopathology
Histopathological changes of bone can be evaluated by staining the transverse section with hematoxylin and eosin.
Results

In the present study we found that removal of rat ovaries produces similar changes as that of post menopausal women. The current work is a part study and primariely focused on prominent evaluation parameters for screening of anti osteoporetic activity. study revealed that there is an increase in body weight after ovariectomy as reported in other experimental studies. After ovariectomy, there is a deficiency of estrogen that occurs. This suggests that estrogen plays a very important role in lipid metabolism. Estrogen insufficiency is thought to be largely responsible for an increase in adiposity during menopause. Estrogen Replacement Therapy in postmenopausal women does not display the characteristic pattern of abdominal weight gain usually associating with menopause.

A marked atrophy of the uterus has been used as evidence of the success of ovariectomy for estrogen directly affecting uterine weight. Indeed; ovariectomy resulted in significant decrease in uterine wet weight. ovariectomized rats developed similar changes to those seen in postmenopausal women as indicated by increased serum ALP, thus suggesting that ovariectomy increased the bone turnover rate in animals. Serum ALP is an important biochemical marker of bone formation. The level ALP increases in osteoporosis and other bone metabolic disorders. Ovariectomy has a more profound influence on
cancellous bone than on cortical bone and considered as a promising model to evaluate the anti-osteoporosis activity in experimental animals.

**Discussion**

It is well-known that estrogen deficiency is an important risk factor in the pathogenesis of osteoporosis. Ovariectomy in the rat results in an increase in bone turnover rate and significant loss of trabecular bone such as the proximal femur, vertebral bodies and the metaphysis of long bones. The micro-architectural alteration in cancellous bone is similar to those observed in postmenopausal and age-dependent (Miller 1988; Kalu 1984).

Mechanical support is the main function of the skeleton. Osteoporosis damages mechanical property of the bones and typically exposes elderly people, especially, to hip, spine and distal forearm fractures. Mechanical bone strength is a reflection of the collective components of bone mass and architecture and represents the best overall index of bone quality. The bone strength is a result of the collagen and crystallized mineral salts (Zhen-Guo 2009). Ovariectomy leads to decreased trabecular bone volume, bone mineral density, and strength in rats. Thus a model for osteoporosis which could clearly reveal the loss of bone mechanical strength is desirable. Since the same effect is seen in the post-menopausal women, the ovariectomized rat model was selected for this study (Junko 2004; Yan Zhang 2007; Donahue 1988).

A potential alternative to estrogen is Raloxifene (Evista), which was approved by the FDA for use in the prevention of osteoporosis in postmenopausal women (Peak 1993). This drug is classified as a Selective Estrogen Receptor Modulator (SERM) belonging to the benzothiophene class of compounds (Kalu 1984; Guyton 2006). Studies suggest that Raloxifene has a mixed agonist (bone and lipid)/antagonist (uterus and breast) profile, which is unique, and these mixed properties are potentially quite useful. In both short- and long-term studies, Raloxifene effectively prevented bone loss in ovariectomized rats in a fashion histomorphologically similar to that of 17β-estradiol while being potently antiuterotrophic. Like Raloxifene, Tamoxifen has both estrogen agonist and antagonist activities and has a favorable effect on bone markers and serum lipid profiles (Goldfrank 1999).

**Conclusion**

From current study, it is concluded that the ovariectomy in Wistar rats produces a similar pathological condition in postmenopausal women. Ovariectomy in animals causes lack of estrogen and affects major bone that can be revealed by a change in physical parameters.

**Conflict of interest**

Conflict of interest declared none.

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