

Changes in femoral bones from rats under osteoporosis preventive treatments

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Bone is a dynamic tissue which is formed and destroyed continually under the control of hormonal and physical factors. An imbalance between these processes may result in loss of bone mass (osteopenia) along with micro-architectural deterioration of the skeleton, leading to bone fragility and an increased risk of fracture (osteoporosis).

Osteoporosis is a disease that deteriorates the bone due to, among other things, a failure in the normal hormonal function and it is mainly associated to post-menopausal complications. This project studies the effects of different therapies based on hormonal supplementation applied to prevent or treat this disease. Four groups of bones (healthy, osteoporotic without treatment, and osteoporotic subject to two different treatments) have been analyzed with nuclear microscopy and the concentration and distribution of certain elements suggest a change in bone density. These bones are now being analyzed with DiTo at HasyLab [1] in order to image this loss of bone mass, with the aim of correlating the apparent changes in density to the changes in elemental distribution and concentration. The final scope is to establish the effects of these hormonal replacement therapies on bone remodelling in order to better weigh the benefits of steroids supplementation for osteoporosis treatment.

Female Wistar rats weighing approximately 250 g each have been used in this study, which is being conducted following the principles for the care and use of laboratory animals according to the European Union guidelines. The animals were exposed to constant periods of light and darkness and to a standard diet (Sanders S.A., Madrid, Spain). A total of six animals constituted the control or basal group (C). The others were ovariectomized, in order to induce osteoporosis, and divided into three groups 15 days after ovariectomy. The first group of five animals received a placebo (O), another group of four animals was treated with 17- β oestradiol (E) and the other six animals were treated with micronized progesterone (P). After one month of treatment, all animals were sacrificed and their femoral bones were excised. For the study, 1 mm thick diagonal cross sections of the femoral bones were cut using a microtome. The orientation of the cut was chosen in order to obtain slices comprising the three different femur regions: epiphysis (EPI), femoral neck (NEC) and diaphysis (DIA). The sections were freeze-dried and examined by optical microscopy in order to evaluate any possible alterations due to the sample manipulation.

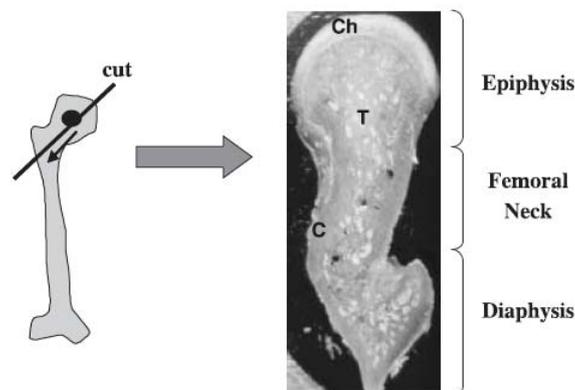


Figure 1: Femoral bone diagonal cross-section where epiphysis, femoral neck and diaphysis are distinguished. Bone structures are indicated: c, cortical bone; T, trabecular bone; Ch, cartilaginous head.

Nuclear microscopy results showed significant elemental differences between control and osteoporotic samples basically related with Ca and P levels [2, 3]. The results seem to indicate that progesterone affects the contents of P, Ca and trace elements in bone after ovariectomy. Nevertheless, a complete recuperation of bone elemental contents to the levels observed in control rats could not be reached. Oestrogen supplementation did not improve the ovariectomy status although an increase in Ca/P ratio was observed in cortical and trabecular bone and especially in the EPI and NEC femoral regions. The associated variability of trace elements according to bone type and region together with the variations observed in P and Ca following hormonal supplementation suggest a recovery from bone loss, possibly a delay in bone resorption and/or an acceleration of new bone formation.

With X-ray tomography, the volume percentages of cortical and trabecular bone in each of the three femoral areas (DIA, NEC and EPI) in the four types of samples analyzed can be obtained. A clear difference can be observed between healthy and osteoporotic samples, particularly in the trabecular bone. Both treatments show an improvement in comparison to the non-treated osteoporotic bone, although the healthy volume percentages are not always reached by either of the treatments, what is more evident in the DIA region.

The tomography analyses performed so far have yielded qualitative results which agree with the conclusions achieved from the nuclear microscopy study. In particular, the deterioration of the bone after ovariectomy is evident. The estimation of the volume percentages of the trabecular and cortical bone show this deterioration of the osteoporotic bone, as well as the recuperation of the osteoporotic bone after treatment, although without reaching the healthy state. The higher proportion of trabecular bone in osteoporotic samples with oestrogen supplementation in comparison to the one with progesterone supplementation may be linked to the higher Ca to P ratio observed in the first one with nuclear microscopy.

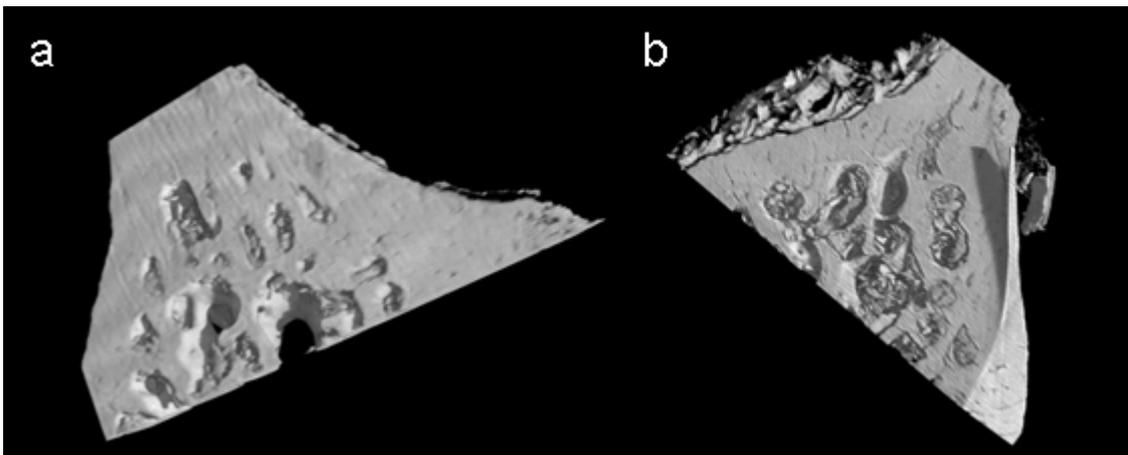


Figure 2: Rendered images of the reconstructed data from the diaphysis region of an (a) osteoporotic bone without treatment and (b) osteoporotic bone treated with progesterone.

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References

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