Extracorporeal Shock Waves, Alone Or Combined With Raloxifene, Reduce Bone Loss In A Rat Model Of Osteoporosis Induced By Ovariectomy

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Osteoporosis is a skeletal disease characterized by the loss of bone mass and strength, inducing to a deterioration of bone micro-architecture and increased fracture risk (Sambrook et al. 2006). Selective estrogen-receptor modulators, including raloxifene, are approved for the prevention and treatment of postmenopausal osteoporosis even if they reduce only vertebral fracture with no effect on hip and other non vertebral fractures (Reginster 2011). Shock wave (SW) therapy seems to be a possible alternative to the standard pharmacological approach, based on its anabolic and anti-inflammatory effect (van der Jagt et al. 2011; Mariotto et al. 2009).

The purpose of this study was to evaluate and compare serum parameters and tissue markers related to the osteoporotic process in ovariectomized (OVX) animals after repeated SW applications, alone or in combination with raloxifene.

Female rats were bilaterally ovariectomized to reproduce a model of mild obesity and osteoporosis. The sham-operated (SHAM) animals were subjected to the same general surgical procedure as OVX groups except that ovarian excision. Sixteen weeks after surgery, rats were divided into five groups (n=6): 1) SHAM; 2) OVX; 3) OVX rats treated with SW application at the anterolateral side of the right hind limb (one session a week, at 3 Hz with an EFD of 0.33 mJ/mm^2, Duolith®) (OVX+SW); 4) OVX rats receiving raloxifene (5 mg/kg/die) *per os* for five days a week (OVX+RAL); and 5) OVX rats treated with SW in combination with the SERM (OVX+SW+RAL). Treatments were performed for five weeks. At the end of experimental time, sera and bone tissues (femurs, tibiae and vertebrae) were sampled for following biochemical and histological analysis.

Ovariectomy (21 weeks) led to a significant increase in body weight and fat mass. RAL significantly prevent these parameters alone or in combination with SW. The reduction in femur weight in OVX was partially prevented by both treatments and normalized by SW+RAL, this effect was also evident in contralateral non-treated femurs indicating a systemic effect of this combination. The efficacy of treatments in restoring vertebrae architecture was more evident in histomorphometric analysis, where SW therapy was more effective than RAL in improving trabecular architecture and osteoid amount, first step of new bone formation. Serum parameters involved in bone remodeling (alkaline phosphatase) or osteoblast proliferation (parathyroid hormone) were examined. All treatments and in particular SW+RAL were able to restore the levels of these parameters altered by OVX. Finally we focused on various genes and transcription factors which regulate osteoclastogenesis and osteoblastogenesis (RANKL, OPG, cathepsin k, Runx-2, BMP-2). We demonstrated that SW, RAL and their combination, reduced RANKL/OPG ratio both in serum and in tibiae, remarkably increased in OVX, resulting in osteoclastogenesis inhibition. SW+RAL significantly reduced cathepsin k tibiae levels, a proteolytic enzyme over-expressed by an intense osteoclasts activity. All treatments significantly increased Runx-2 and Bmp-2 expression, evidencing an increase in osteoblastogenic activity.

In conclusion, our study demonstrate the beneficial effects of SW therapy, alone or in combination with raloxifene, restoring the balance between bone formation and resorption, and identifying SW therapy as an innovative strategy to limit the progression of osteoporotic process.

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