BIOMECHANICAL AND HISTOLOGICAL EVALUATION OF ESTROGEN, RALOXIFEN, VITAMIN K2 AND THEIR COMBINATIONS IN THE TREATMENT OF OSTEOPOROTIC BONE

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INTRODUCTION

In this study, **Estrogen**, the most common hormone replacement therapy (HRT) agent, was used as single and combined with **Raloxifen**, a SERM type **osteoporosis** drug. Despite their high clinical uses, they have not been tried before, in combination. They act as agonist of each other in uterus and mammary glands. Therefore, it was expected to prevent HRT side effects by using the combinations while enhancing the healing of osteoporotic bone. As a third agent, **Vitamin K2** was chosen to be applied alone or in combination with Raloxifen. Although the recent studies mentioned the effects of Vit K2 on bone, its rebuilding and repair effect was not completely established. Hence, Vit K2-Raloxifen combination was applied and compared with the other groups as a new treatment. To understand the single and combined effects of these three agents, mechanical properties of bone was studied with both **strain gage** and **bending** theory methods on **ovariectomized** rat model**.** Also, histological evaluations of bone and uterine tissues were carried out together with blood analysis.

METHODS

Rats (56) were divided into 7 groups as Estrogen (E), Raloxifen (R), Vit K2 (K), Estrogen and Raloxifen (E+R), Vit K2 and Raloxifen (R+K), Ovariectomized controls (C), and Sham operated group (S). All drug groups were ovariectomized before the treatments. Drug treatments were started three months after the surgery, and continued for 12 weeks. Estrogen and Vit K2 were applied by subcutaneous injection, while Raloxifen was administered orally. Uterus and right tibia were taken for histological analysis. Left tibia and both femora were wrapped in saline soaked gauze sponge and stored at -20^{o}C until DEXA measurements and biomechanical testing.

RESULTS AND DISCUSSION

All the treatments have resulted in numerically higher values for mechanical properties of femora and even significantly better values for tibia when compared to untreated controls (Table 1). The combined therapies performed better than the individual administrations and both of the controls. The modulus of elasticity was calculated from the strain gage data and compared with that obtained from the beam deflection formula. Computed moduli were consistent with literature. [1-3] However, there was a significant difference (about 13 times) between the values obtained from the two approaches. Similar difference was also observed and explained in another study for healthy rat tibia [2].

Table 1: Comparison of mechanical properties for tibia

TIBIA	ULTIMATE STRENGTH (MPA)	MODULUS OF ELASTICITY (GPA)	ENERGY ABSORBED (NMM)
C	**100.11***	3.26	46.08
S	176.71	5.53	51.13
E+R	202.22	5.87	**73.59 ***
E	174.95	4.28	63.63
R	187.96	4.44	65.59
K	170.09	5.12	59.58
R+K	189.94	5.30	57.77

* Statistically different from other groups

Biochemical analysis of blood showed an increase in bone formation (ALP activity) compared to controls. The highest ALP activity among all was observed in R group. Quantitatively, E+R had the maximum BMD values for proximal and distal of femur and tibia. As a general trend, treatment groups had better BMD values than the ovariectomized controls. Histologically, enlargement in uterus, and degeneration in connective tissue were highly observed in E group. However, such changes were less in combined groups and in the groups not involving estrogen. This implied that the adverse effects of estrogen on uterus could be reduced by using R.aloxifen.

CONCLUSIONS

Antiresorptive agents are effective in osteoporosis prevention and treatment, but their combination was found to be much better mechanically, histologically and densitometrically than their separate usage. Strain gage data was thought to be more informative than the deflection data while determining the mechanical properties of rat femur under three point bending. Vit K2 was found to be an important factor in increasing the strength of bone especially at high stresses.

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