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The Effects of Zoledronate and Sleep Deprivation on the Distal Femur Trabecular Thickness of Ovariectomized Rats: Application of Different Statistical Methods

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Introduction

Osteoporosis is a disease of the skeletal system in which bone is compromised leading to increased risk of fracture [1]. One in three women will experience a fracture due to osteoporosis in their lifetime, with postmenopausal women being the most susceptible [1]. One of the main intrinsic factors that increase the risk of osteoporotic fracture is women having a longer bone density in the microarchitecture of bone [1]. Since trabecular bone is 3-4 times more metabolically active than cortical bone [2], distal femur trabecular thickness was used as the parameter for bone quality in this study. Data suggested that 25% of Americans reported insufficient sleep (>6 hr over more than 15 days/month) [3]. Heightened adverse physiological effects, including lowered BMD and decreased bone quality, resulting from sleep deprivation were identified in menopausal females [4], therefore, this study aimed to incorporate sleep-deprivation as a variable in bone health. Bisphosphonates, such as Zoledronate, are a common treatment for osteoporosis. Zoledronate attempts to inhibit osteoclastic activity by blocking a portion of the mevalonate pathway through the inhibition of farnesyl pyrophosphate synthase which restricts the prenylation of certain GTPases [5]. The purpose of this study was to evaluate the consequences of sleep deprivation on bone metabolism with and without the protective effects of Zoledronate in sleep-deprived, estrogen-deficient rats.

Methodology

Ovariectomized Wistar female rats (n=31) were received from the Wistar Institute and given a 1-week adjustment period in standard conditions. Standard conditions included a 12-hour light/dark cycle and food and water provided ad libitum. The rats were randomly assigned into 4 groups including a Control (C, n=4), Zoledronate (Z, n=9), Sleep-deprived (SD, n=9), and Zoledronate + Sleep-deprived (SDZ, n=9). The C and SD groups were given a one-time intravenous injection of 0.45mL of 0.9% saline. The Z and SDZ group received a one-time intravenous injection of 50ug/kg body weight of 10% Zoledronate. After the injection, the 5-week protocol was started. The C and Z groups were kept in standard housing. However, the SD and SDZ groups were housed in MMP tanks (Figure 2) which prevented sleep for 18 hours, then moved to the sleep chambers for 6 hours. After 5 weeks, the rats were exsanguinated and the left and femora were collected, wrapped in saline-soaked gauze, stored at -80°C, and shipped to Novartis Institute for Biomedical Research where high-resolution micro-computed tomography scans were performed (Figure 2). Statistical analysis included ANOVA, Tukey, Bonferroni, and Kruskal Wallis tests as well as the creation of an interaction graph using ANCOVA in R Studio.

Results

The purpose of this study was to evaluate the trabecular thickness using a commonly prescribed bisphosphonate in attempts to explore potential advantages in sleep-deprived, ovariectomized rats. An analysis of covariance (ANCOVA) model was used to predict trabecular thickness with treatment group as a main factor and housing as a covariate, including a factor for the interaction of treatment and housing. There was a significant treatment and housing effect on trabecular thickness (each p<0.0001), with the interaction also being a significant (p=0.0078), suggesting treatment affected mean trabecular thickness differently by which housing rats were in. Overall the model achieved an R² of 0.612. Assumptions of normality of the residuals were not investigated further, but a non-parametric Kruskal Wallis test was run to verify the pattern of the ANCOVA results (p<0.001). Further investigation using Tukey (confirmed with Bonferroni) multiple comparisons tests revealed there were significant differences between SDZ and C (p<0.0001), SD and SDZ (p<0.0001), and the Z and SDZ groups (p<0.0001).

Discussion

Using more robust statistical analyses on previously gathered data has revealed a possible physiological interaction between the amount of sleep and the use of Zoledronate. Further studies are needed to determine the mechanism behind the physiological interaction, but results could have a profound impact on the use of Zoledronate in modern society where many people are getting less sleep because of work demands.

Citations


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