LONG-TERM ESTROGEN REPLACEMENT THERAPY DECREASES OSTEOARTHRITIS SEVERITY IN Cynomolgus MONKEYS

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Introduction: Estrogen deficiency occurring with menopause has been associated with an increased incidence and severity of osteoarthritis (OA) in older women. Most observational studies suggest that postmenopausal women taking estrogen replacement therapy (ERT) have a reduced risk of radiographic hip and knee OA [1]. The purpose of this study was to determine the effects of long-term ERT on the progression and severity of knee OA in surgically postmenopausal (bilaterally ovariectomized) female cynomolgus monkeys. This nonhuman primate model of OA is extremely useful due to the close phylogenetic relationship between monkeys and humans, and the similarity of the disease to that occurring in humans [2]. In addition, there are distinct advantages to studying this disease in monkeys, since human studies are complicated by many factors which may modify the effects of estrogen, including the potential effects of concurrent progesterone use.

Materials and Methods: The study included 120 feral adult female cynomolgus macaques that were bilaterally ovariectomized to simulate menopause, and randomly divided into two age and weight-matched treatment groups using a stratified randomization scheme: 1) control group (n=60); and 2) Premarin™-treated group (Premarin™ [conjugated equine estrogens], Wyeth-Ayerst Laboratories) (n=60). The treatments were given for three years. Tissues from 106/120 monkeys (control [n=52], Premarin™-treated [n=54]) were available for evaluation in this study. At necropsy a mid coronal section of the right tibial plateau was decalcified, embedded in paraffin, sectioned at 6 µm and stained with toluidine blue. The histological sections were randomized and relabeled to blind the evaluator to the treatment groups. Each lateral and medial tibial plateau was graded using a semiquantitative histological grading scale that included assessment of articular cartilage fibrillation, loss of toluidine blue staining and subchondral bone thickness. In addition, vessels crossing the tidemark, chondrified vessels, chondrocyte clones, osteophytes and tidemarks were counted. The perimeters of the articular cartilage, calcified cartilage and subchondral bone were measured using the Osteomeasure histomorphometry system (Osteometrics, Inc.), and widths and areas were calculated.

The data were summarized by principal components analysis, and the resulting factors and individual variables were compared using ANOVA, and ANCOVA using age and weight as covariates. As in previous studies [2] the scores and measurements revealed more severe disease in the medial plateaus than the lateral, therefore only the medial data were evaluated statistically.

Results: Overall, the histological lesions of OA were mild to moderate, which was expected since the animals were relatively young at the time of necropsy (mean age = 11.9 years, range = 9.6-15.8 years). Principal components analysis identified four factors that explained 71% of the variability in the data. The differences in subchondral bone thickness were explained by factor 1 (weighted primarily by subchondral bone width and subchondral bone area). Variability in articular cartilage thickness was explained by factor 2 (weighted primarily by articular cartilage width and area). The differences in OA severity were described by factor 3 (weighted primarily by articular cartilage fibrillation score, loss of toluidine blue staining score and number of chondrocyte clones). Factor 4 described the differences in calcified cartilage thickness (weighted primarily by calcified cartilage width and area). Factor 1 scores (weighted by subchondral bone thickness) were significantly higher in the Premarin™ group compared with the control group (p < 0.05) (mean subchondral bone thickness: Premarin™ group = 395 µm; control group = 351 µm). Factor 2 scores (weighted by articular cartilage thickness) were not significantly affected by treatment (data not shown). Factor 3 scores (weighted by variables describing OA severity) were significantly higher in the control group compared with the Premarin™ group (p < 0.05), and the effect remained significant when adjusted for age and weight (Table 1). When adjusted for group and weight, factor 3 was also significantly affected by age (p < 0.01) (Table 1). Factor 4 (weighted by calcified cartilage thickness) was not significantly affected by treatment in either analysis (data not shown). Although not statistically significant, the average number of osteophytes was lower in the Premarin™ group (0.88 ± 0.57) than in the control group (1.34 ± 0.60).

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Discussion: To our knowledge, this is the first long-term study to demonstrate the protective effect of ERT at the histological level in an animal model. Long-term Premarin™ treatment significantly decreased articular cartilage lesions of OA. Although ERT resulted in a mild increase in subchondral bone thickness, it also tended to decrease osteophyte numbers.

There is little known regarding the pathogenesis of early OA and, to our knowledge, no previous controlled studies have been done regarding the role of estrogen in the early stages of the naturally occurring disease. Although there are risk factors for OA that are more pronounced than estrogen deficiency, such as age and weight, they are not easily modifiable. Our results, coupled with the results of previous epidemiological studies [1], provide strong evidence that ERT may be an effective way to decrease the severity of OA in postmenopausal women.

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References:

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