Effects of Gender and Hormones on Posttraumatic Osteoarthritis

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Disclosures:

Introduction: After menopause, the relative incidence of osteoarthritis in estrogen-deficient women increases, and this effect appears to be mitigated with estrogen replacement therapy (1; 2). Clinically, joint injury risk is disproportionately higher in young female athletes, and the pathoetiologies of posttraumatic osteoarthritis are incompletely understood. A meta-analysis found evidence of a relationship between ovariectomy and osteoarthritis in animals, with estrogen replacement reversing this effect (3). Most preclinical research of posttraumatic osteoarthritis uses male animals. To evaluate the role of estrogen status in the development of posttraumatic osteoarthritis, we induced osteoarthritis in male and female rats by surgically destabilizing the medial meniscus (DMM). We hypothesized that DMM in an ovariectomized rat would result in more pronounced osteoarthritis than that seen with DMM alone.

Methods: All animal experiments were approved by the local Institutional Animal Care and Use Committee. In accordance with Osteoarthritis Research Society International (OARSI) guidelines, 3-month-old Lewis rats were used (4). Female rats were ovariectomized (OVX) or left ovary intact (OVI) 2 weeks prior to DMM of the right knee or sham surgery. Urine was collected at baseline and 2 weeks thereafter and was analyzed for CTX-II by ELISA (Uscn Life Science Inc., Wuhan, Hubei, China) and total protein by BCA protein assay (Pierce, Rockford, IL). Rats were sacrificed 8 weeks post DMM. Knees were collected intact and fixed in 10% formalin for 3 days, decalcified, processed, embedded in paraffin, and sectioned coronally at 12 µm. Sections were stained with hematoxylin and eosin, randomized, and scored by 2 blinded investigators using the OARSI scoring system (5). Serum estradiol at the time of sacrifice was measured with EIA (Cayman Chemical, Ann Arbor, MI) and total protein with BCA protein assay. This study was powered to detect a 1.4 difference between OVX and OVI within the sham treatment with 80% power at the 5% significance level assuming a 1.0 standard deviation of the differences between left and right pairs. A repeated-measures analysis of covariance was used to test for DMM and ovariectomy effects over time on protein-normalized CTX-II. The baseline CTX-II was used as a covariate. An analysis of variance model, including a random subject effect, was used to test for these same effects on the average histology score. Comparisons were made between left and right sides. A Mann-Whitney test was used for the between-group comparison on protein-normalized estradiol.

Results: Serum estradiol levels were significantly lower following ovariectomy (Figure 1). In females, DMM resulted in higher histologic scores and thus an increase in cartilage degradation in the injured limb compared with the uninjured limb in both OVX and OVI rats (Figure 2, top panel). Increased cartilage degradation was not observed in the sham-surgery limb compared with the contralateral limb. OVX rats did not have increased cartilage degradation compared with OVI rats. In males, DMM resulted in an increase in cartilage degradation in the injured limb compared with the uninjured limb (Figure 2, bottom panel). Again, sham surgery did not increase cartilage degradation. CTX-II levels were unaltered at all timepoints in all treatment groups across sexes (Figure 3).

Discussion: DMM caused cartilage degradation in both male and female rats by 8 weeks after surgery. The combination of ovariectomy and DMM did not yield more pronounced osteoarthritis, as we had hypothesized. In fact, ovariectomy alone did not result in cartilage degradation, which is inconsistent with some previous reports (3). In a mouse model, ovariectomy actually decreased cartilage degradation due to DMM-induced osteoarthritis (6). Differences in study design, such as strain, species, and age, may account for these inconsistencies.

Significance: In this study, we evaluated the role of estrogen in an induced posttraumatic osteoarthritis rat model, laying the foundation for future studies on the development of osteoarthritis in estrogen-deficient females. From a translational perspective, posttraumatic osteoarthritis is becoming increasingly more prevalent among women. The investigation of the effects of estrogen status appears to be important; however, the role of estrogen levels on the development of osteoarthritis has not been identified in this animal model.

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Figure 2. Histological scores of articular cartilage in female (top) and male (bottom) rats. Data presented as mean±SE. *P<0.01, **P<0.001

Figure 3. CTX-II levels in urine across timepoints in female (top) and male (bottom) rats. Data presented as mean±SE.