Apoptosis in Articular Cartilage After Injury is Dependent on Estrogen Status in Experimental Osteoarthritis

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Disclosures:

Introduction: Epidemiological studies have shown that there are significant gender differences in both the prevalence and the clinical manifestations of osteoarthritis (OA) (1). OA appears to be more prevalent in men than women before the age of 50. With the onset of menopause in women, both the prevalence and severity of OA in women increases (1). Small animal models of OA have shown that female mice develop less OA than males, and that ovariectomized (OVX) female mice develop worse OA than female mice with intact ovaries (2). One hypothesis is that estrogen may be a chondro-protective molecule. There has been increasing evidence in recent years that suggest sex hormones and estrogen in particular may be important in maintaining homeostasis of cartilage as well as other articular tissues (3). Notably, the alpha- and beta- receptors for 17-β estradiol (E2) are both expressed in joint chondrocytes of both male and females, which suggest direct effects on chondrocytes (4). However, the exact cellular and molecular mechanism(s) of E2 in OA chondroprotection are unknown. It is our hypothesis that estrogen acts to inhibit the early chondrocyte apoptosis event which occurs shortly after OA-inducing soft tissue injury.

Methods: OA was induced in the right knees of all female mice using destabilization of the medial meniscus (DMM) surgery at 10 weeks of age. In addition, the following conditions were performed: OVX or sham ovariectomy and/or E2 injections were also added to the DMM female mice (OVX-DMM) as follows: 1) double OVX were performed on mice 2 weeks prior to the DMM surgery (n=3). 2) double OVX were performed on mice 2 weeks prior to DMM and treated with 17β-estradiol 3- benzoate (Sigma) injections (s.c.) of 1.92mg/kg every 4 days following OVX (n=3) 3) In the control group, sham OVX were performed 2 weeks prior to DMM (n=3). All mice were sacrificed at 4 days after DMM surgery. Right knees were harvested, processed and sectioned at 6 um intervals. The TUNEL immunofluorescence assay was used to assess for apoptotic cells on 16 sequential coronal sections spanning 385 +/- 15 um of each knee representing the central weight bearing region of the tibial plateau, giving a total of 48 data points per experimental group. TUNEL images were digitally captured. Outcome measures included: 1) total number of apoptotic chondrocytes in the superficial zone 2) cartilage lesion area 3) lesion width 4) lesion height. Outcome measures were processed and assessed by ImageJ (rsbweb.nih.gov). Comparison of the experimental and control groups was performed by T test and 95% CI was calculated. A p-value of less than 0.05 was considered to indicate significant differences.

Results: Mice with intact ovaries developed significantly less apoptosis (TUNEL positive cells) than OVX-DMM (ovariectomized) females (34% reduction; p<0.01) (Table 1). There were significantly less total apoptotic cells in the superficial-middle zone as well as less apoptotic cells in a clearly defined lesion area within the superficial-middle zone. Additionally, mice with intact ovaries developed smaller cross sectional OA lesion areas (48% reduction; p<0.01), as well as smaller width (36% reduction; p<0.01) and height (24% reduction; p<0.01) of the lesion margins compared to OVX-DMM mice. There was significantly less apoptosis (31% reduction; p<0.01) in OVX-DMM mice with E2 injection as compared to OVX-DMM mice without E2 injection (Table 1). There was no significant difference in apoptosis between the intact ovaries group and OVX-DMM + E2 treatment group. E2 treatment did have a modest but significant reduction in lesion width (9% reduction; p<0.05) (Table 1).

Discussion: Our analysis of articular cartilage showed that apoptotic chondrocytes appear as an early event following soft tissue injury to the knee (DMM) in female mice. In mice with intact ovaries, there were significantly less number of apoptotic cells following DMM compared to OVX-DMM mice. The resulting lesion size was also significantly smaller. In addition, in OVX-DMM females with estrogen replaced, there were also significantly less number of apoptotic cells following DMM as compared to OVX-DMM mice with no treatment. There was however not a significant reduction in the size of the lesion that developed. The number of apoptotic cells were similar in the OVX-DMM with estrogen treatment group and the non-OVX-DMM group. These data suggest that estrogen, which is mainly produced in the ovaries, may have direct anti-apoptotic effects after an induction of injury to the joint. It has recently been observed in the literature that estrogen has chondroprotective effects and is important in the homeostasis of joint maintenance at many crucial levels through several complex molecular mechanisms [5]. We propose that one mechanism through which this occurs is via direct anti-apoptotic actions of estrogen on chondrocytes after an injurious event has occurred. We also propose that since early estrogen intervention in OVX-DMM mice can reduce apoptosis in OA mice, early medical interventions following knee injury can be made that may alter the progression of later OA stages.
Significance: It has been observed in the literature that estrogen has chondroprotective effects, we propose that one mechanism through which this occurs may be direct anti-apoptotic actions on chondrocytes after an injurious event has occurred. We also propose that as apoptosis occurs immediately after an injury has occurred and that estrogen treatment in a OVX-DMM mice can reduce apoptosis to levels similar to that of ovaries intact mice, 1) interventional can be made that can maybe alter the progression of OA development 2) these interventions must perhaps be made in a time specific manner.

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