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Original article

Menisectomized miniature Vietnamese pigs develop articular cartilage pathology resembling osteoarthritis

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ABSTRACT

Animal models have been used to understand the basic biology of osteoarthritis (OA) and have helped to identify new candidate biomarkers for the early diagnosis and treatment of this condition. Small animals cannot sufficiently mimic human diseases; therefore, large animal models are needed. Pigs have been used as models for human diseases because they are similar to humans in terms of their anatomy, physiology and genome. Hence, we analyzed articular cartilage and synovial membrane pathology in miniature Vietnamese pigs after a unilateral partial menisectomy and 20-day exercise regimen to determine if the pigs developed pathological characteristics similar to human OA. Histological and protein expression analysis of articular cartilage from menisectomized pigs revealed the following pathologic changes resembling OA: fibrillation, fissures, chondrocyte cluster formation, decrease in proteoglycan content and upregulation of the OA-associated proteins MMP-3, MMP-13, procaspase-3 and IL-1β. Moreover, histological analysis of synovial membrane revealed mild synovitis, characterized by hyperplasia, cell infiltration and neoangiogenesis. Pathological changes were not observed in the contralateral joints or the joints of sham-operated pigs. Further studies are required to validate such an OA model; however, our results can encourage the use of pigs to study early stages of OA physiopathology. Based on their similarities to humans, pigs may be useful for preclinical studies to identify new candidate biomarkers and novel treatments for OA.

Introduction

Osteoarthritis (OA) is a chronic, degenerative and incapacitating disorder characterized by articular cartilage degeneration, mild synovitis and peri-articular/subchondral bone alterations [1]. OA is a common disease, affecting millions of people worldwide. In 2005, data from the Centers for Disease Control and Prevention (CDC, USA) estimated that 27 million adults in the United States were affected by OA (http://www.cdc.gov/arthritis/basics/osteoarthritis.htm). The probability of developing OA may increase with age; however, genetics, gender, obesity, abnormal joint shape and joint injuries are also common risk factors [2,3].

One pathological hallmark of OA is the progressive degradation of articular cartilage. Articular cartilage is a specialized connective tissue primarily composed of a network of collagen (II, IX, and XI) and proteoglycans (mostly aggrecan). Cartilage degradation is induced by mechanical stress on joints and the enzymatic activity of matrix metalloproteinases (MMPs-1, -3, -13) and aggrecanases (ADAMTS-4 and -5) [4,5]. These enzymes degrade the molecular components of the cartilage matrix. Moreover, the expression and activity of MMP and ADAMTS are induced by pro-inflammatory cytokines such as IL-1β and TNF-α [6,7]. These cytokines play a key role in OA development by directly inhibiting the expression of cartilage-specific extracellular matrix genes, such as aggrecan and collagen type II [8–10], and upregulating a range of catabolic genes, including those encoding matrix-degrading proteases [5,10–12]. As a consequence, the mechanical and biochemical insults cause structural alterations throughout the cartilage architecture. These alterations include fibrillation, changes in the amount and composition of matrix proteins, development of cell clusters and phenotypic variability of chondrocytes, including programmed cell death [13–16].

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Although OA is considered a non-inflammatory joint disease [1], it is widely accepted that synovial inflammation is a factor that contributes to the deregulation of chondrocyte function and loss of articular cartilage matrix, since synoviocytes and infiltrating mononuclear cells overexpress catabolic molecules such as pro-inflammatory cytokines and MMPs [17]. Currently, there is no cure for OA, and the most common treatments only ease the symptoms.

In biomedical research, experimentation is essential for identifying the mechanisms and basis of diseases. However, many scientific studies cannot be performed in humans; therefore, animal models are required. In fact, animal models have been used to elucidate the basic biology of OA and characterize candidate biomarkers for OA diagnosis and treatment [18–20]. However, in order to facilitate the translation of results from animals to humans, larger and more similar animal models are needed.

Pigs have been used as models for several human diseases. They are most similar to humans in terms of their anatomy, neurobiology, cardiac vasculature, gastrointestinal tract, and genome [21–24]. In addition, technological advances in cloning and transgenics have permitted the application of genetically modified and cloned pigs in translational research [22,24]; thus, pigs have become the non-rodent biomedical model of choice [25]. In rheumatology and orthopedics, pigs have been used as animal model [17] and source of articular chondrocytes to study the physiopathology of OA [see, for example, 26, 27]. Notably, pigs are useful models for studying the repair and regeneration of focal cartilage defects [reviewed in 28, 29]. Furthermore, the porcine joint size, weight-bearing requirements, and cartilage thickness mimic human characteristics better than those of small animal models [30,31]. Therefore, we used miniature Vietnamese pigs to evaluate the changes in articular cartilage and synovial membrane after a menisectomy and 20-day exercise regimen. Our results showed that juvenile pigs resembled human OA according to the following pathologic characteristics in their articular cartilage: fibrillation, fissures, chondrocyte cluster formation, decrease in proteoglycan content and upregulation of the OA-associated proteins MMP-3, MMP-13, procaspase-3 and IL-1β. Moreover, histological analysis of synovial membrane revealed mild synovitis, characterized by hyperplasia, cell infiltration and neoangiogenesis. These results show the suitability of using pigs to study the physiopathology of OA. Pigs can also be useful for identifying new candidate biomarkers and novel treatments in preclinical studies.

Materials and methods

Animals

All procedures used for animal care were approved by our institutional *ad hoc* committee and performed according to the animal facility regulations and the Mexican official regulatory guideline NOM-062-ZOO-1999. Nine-week-old (eleven castrated males and nine females, average weight of 8.8 ± 2.5 kg), skeletally immature miniature Vietnamese pigs (*Sus scrofa domesticus*) were used. Pigs are skeletally immature until they reach 6 years of age [32]; therefore, the twenty pigs used in this study were considered juvenile.

Castration is a regular procedure for pigs and it is performed to reduce stress and male aggressiveness; therefore, the procedure allows a better handling and housing of animals.

The pigs were bred under specific pathogen-free conditions and had free access to water. Balanced food (Carnerina No. 1, 600 g daily per pig; Malta Cleyton, MX) was given to the pigs in the morning and in the afternoon.

All surgical procedures were carried out in pigs anesthetized with an intravenous injection of azoperone (2 mg/kg) followed by a mixture of tiletamine and zolazepam (1.5 mg/kg). At the end this study, animals were anesthetized and then killed by bleeding.

Knee menisectomy

The experimental surgical procedure for the pig menisectomy was based on the unilateral menisectomy reported in rats [33]. Under general anesthesia, a straight, 2-cm incision was made on the medial side of the right knee. Afterwards, the medial collateral ligament and the anteromedial side of the joint capsule were cut to expose the body of the medial meniscus. Then, the meniscus was dislocated with forceps, cut at the level of the medial collateral ligament, and excised. As a control, we included pigs that had sham surgery in which the joint capsule tissue was cut but without performing a menisectomy or a collateral ligament transection.

After surgery, pigs were treated intramuscularly with antibiotics (enrofloxacyn, 2.5 mg/kg) and analgesics (flunixyn-meglumine, 2 mg/kg) until the sutures were removed and the wound was completely healed (approximately 8 days). Daily cures were performed on the operated joints for 6 days to avoid bacterial infection.

Exercise regimen for pigs

The exercise regimen for the pigs began approximately 8 days after surgery. The pigs were guided to cross an 8 cm-high and 1 m-wide wood platform 100 times a day (approximately 20 min) for 20 days. A previous pilot study showed that 20 min of daily walking for 20 days did not affect the joints; therefore, we incorporated the low-impact wood step into the exercise.

Cartilage samples

The medial femoral condyles and medial tibial plateaus of both knees were removed and fixed for 48 h at 4 °C in 4% paraformaldehyde in PBS, pH 7.2. After three washes with PBS, small samples were taken from the weight bearing areas and incubated for 12 h at 4 °C in 10% sucrose in PBS, pH 7.2. Afterwards, the samples were embedded in tissue freezing media (Leica Microsystems, Wetzlar, DE) and immediately frozen at −20 °C. For Western blot studies, the articular cartilage was removed from the medial femoral condyles and tibial plateaus from the right knees and immediately frozen and stored at −80 °C until processed to extract the proteins.

Synovial membrane samples

Samples of synovial membrane were removed from the area below the patella and fixed for 24 h at room temperature in 4% formalin in PBS, pH 7.4. After three washes with PBS, samples were dehydrated in ethanol and embedded in paraffin. Paraffin embedded tissues were sectioned (microtome Jung Histocut, Leica Microsystems) to obtain 5 μm-thick slices, which were mounted on silane-coated slides.

Immunohistochemistry

Frozen samples of condyles and plateaus were cryosectioned in the coronal plane of the tissue (cryostat Leica CM1100, Leica Microsystems) to obtain 8-μm-thick slices, which were mounted on gelatin-coated slides. The sections were hydrated for 10 min in PBS, pH 7.2, and permeabilized with 0.3% Triton X-100 in PBS for 3 min at room temperature. Tissues were incubated for 20 min at room temperature with 1.5% normal horse serum in PBS to block non-specific protein binding. Cartilage slices were incubated for 12 h at 4 °C with the primary antibodies diluted in 0.2% BSA in PBS containing 0.01% triton X-100. The rabbit anti-IL-1β primary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and the