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Contribution of IL-1β, 6 and TNF-α to the form of post-traumatic osteoarthritis induced by "idealized" anterior cruciate ligament reconstruction in a porcine model

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ABSTRACT

Background: It has been noted that anterior cruciate ligament (ACL) injury-induced cartilage degeneration is the key risk factor for post-traumatic osteoarthritis (PTOA). However, whether the cartilage degeneration after ACL injury is caused by inflammation, abnormal biomechanics or both remains largely unknown, as there has been no animal model for separating the two factors so far.

Methods: Eighteen-month-old female mini-pigs were divided into an "idealized" anterior cruciate ligament reconstruction (IACLR) group and a control group ($n = 16$ limbs per group). Real-time PCR, safranine O staining and indian ink staining were performed to verify whether animal models were successfully established or not. Multiple linear regression analysis was used to evaluate the correlation between levels of the inflammatory factors (including interferon [IFN]-γ, interleukin [IL]-1β, IL-4, IL-6, IL-8, IL-10, IL-12 and tumor necrosis factor [TNF]-α measured by the Luminex method) and changes in cartilage histology (quantified by morphological scoring) after surgery.

Results: A significant OA cartilage damage with increased MMP-1, MMP-13 mRNA levels and reduced aggrecan mRNA/protein levels was observed in IACLR groups. As a result, the IACLR gross morphology score was dramatically increased than control. Moreover, IACLR significantly increased the levels of IL-1β, IL-4, IL-6 and TNF-α in the synovial fluid of the knee. Most importantly, a close relationship was found between IL-1β, IL-6 and TNF-α concentrations and morphological score of PTOA, respectively.

Conclusion: These results demonstrated that inflammatory factors are independently responsible for the onset of PTOA.

1. Introduction

During recent decades, the anterior cruciate ligament (ACL) injury rates have dramatically increased with traffic accidents and high energy trauma. It is the consensus that ACL injury can cause secondary degeneration of the articular cartilage and meniscus, and eventually result in traumatic osteoarthritis [1]. Although ACL reconstruction (ACLR) can effectively restore the stability of the knee, many patients still developed into post-traumatic osteoarthritis (PTOA) [2]. PTOA is a special type of osteoarthritis (OA) and a common disease involved in various tissues such as articular cartilage, subchondral bone and synovium. It's a result of the biodegradation and synthesis imbalance of chondrocytes, extracellular matrix (ECM) and subchondral bone [2]. The typical pathological changes of PTOA are articular cartilage destruction and secondary bone hyperplasia, which is the consequence of the comprehensive effect of various kinds of factors. However, the pathological mechanism remains elusive.

It has been reported that cytokines and inflammatory factors (such as interferon [IFN]-γ, Interleukin [IL]s, Tumor Necrosis Factor [TNF]-α, Matrix Metalloproteinase [MMP]s, etc.) play an important role in the pathophysiology of OA [3]. IL-1 and TNF-α exert a key role in stromal degradation and cartilage destruction of OA. It can induce the production of other inflammatory cytokines (such as IL-6, etc.), MMPs and prostaglandin (PG), and reduce the synthesis of type II collagen and

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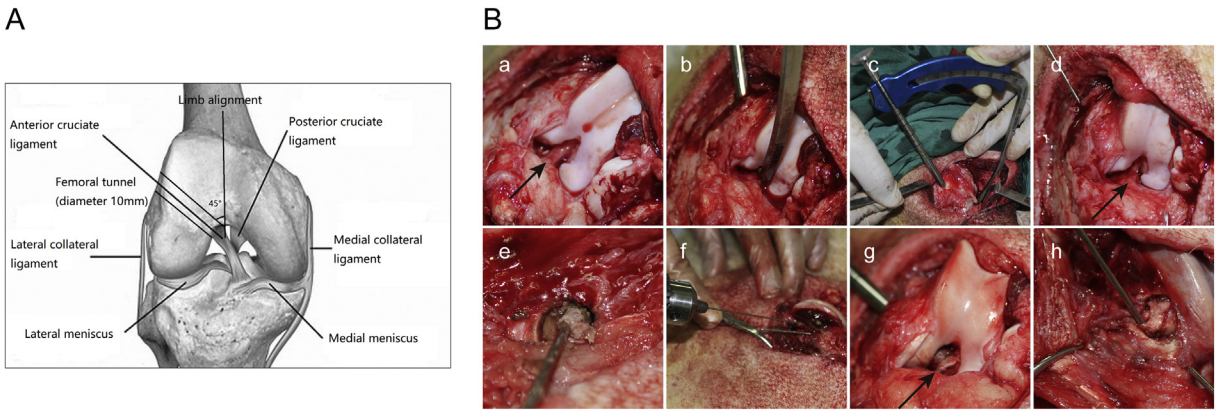

Fig. 1. ACL autograft reconstruction surgery is performed on a mini-pig right hind limb. (A) Schematic of the idealized ACL autograft reconstruction (IACLR) surgery. (B) The surgical process: a. The stifle joint was open and the patella was dislocated to expose the ACL (arrow shown). b. After the ACL femoral insertion point was located, an Aimer Tip Drctr ACL Guide was positioned. c. The crossing angle was 45°. d. One guide pin was drilled into the joint cavity along the ACL Guide. e. A core drill (diameter 10 mm) was used to drill a femoral tunnel from the lateral femoral condyle to the ACL insertion point along the guide pin. f. Before the hollow drill was about to penetrate the femoral tunnel, a Kirschner wire (diameter 1 mm) was drilled along outer edge of the tunnel to prevent the cartilage from splitting. g. The bone core, attached to the ACL (arrow shown), was completely freed. h. After its return, the bone core with ACL autograft was fixed by two Kirschner wires.

aggrecan [3]. These cytokines and inflammatory factors are all closely related to the functional changes of synovial membrane, articular cartilage and subchondral bone in OA. However, it is unclear whether the inflammation independently plays the same role in PTOA of patients whose knee stability is recovered after ACL injury, since no appropriate animal model is available so far. To resolve this gap, we developed an "idealized" ACL autograft reconstruction (IACLR) model using mini-pigs to independently evaluate the effects of the inflammation on cartilage degeneration post-injury. The porcine model exhibit similar physiological and serum biochemical parameters, peripheral bone deposition rate, and trabecular bone density in human. Collagen fibers arrangement in cartilage tissue are also similar to that in human, but rat, rabbit, sheep or goat present a columnar arrange pattern [4]. In addition, the density and concentration of bones in mini-pigs are similar to those in humans [5]. Indeed, domestic pigs are rarely used for animal model, as their large body size and food intake, which is not convenient for experimental operation and feeding management [4]. However, the application of mini-pigs has increased, especially from 1870s [6]. Mini-pigs are currently widely used in many research areas, such as cardiovascular systems, digestive systems, urinary systems, skin burns, stomatology and pharmacological toxicology [6]. Over the past decade, many preclinical orthopedic studies have used them as a model animal for bone research. In terms of morphology [7], bone composition [8], microstructure [9] and remodeling characteristics [10,11], this species is considered to be a close representative of human bone tissue.

In this study, we select aggrecan as the main indicator to verify the success of modeling. Aggrecan, also known as cartilage-specific proteoglycan core protein (CSPCP) or chondroitin sulfate proteoglycan 1, is a major component of the ECM in cartilage tissue, and it also withstand the compressive forces acting on the cartilage in the weight-bearing region. Real-time PCR, safranine O staining and indian ink staining demonstrated a decline in mRNA and protein levels of aggrecan. In addition, our data showed that the expression of inflammatory cytokines (IFN-γ, interleukin IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12 and tumor necrosis factor TNF-α) and matrix metalloproteinase MMP-1 and MMP-13 is increased after IACLR surgery, especially IL-1β, IL-4, IL-6 and TNF-α in IACLR group. We further confirmed the correlation between IL-1β, IL-6, TNF-α and morphological changes of PTOA by linear regression analysis. Therefore, our data indicate that inflammatory factors participate in the pathogenesis of PTOA and may serve as a predictor of the severity of PTOA histological changes.

2. Materials and methods

2.1. Idealized ACL autograft reconstruction surgery

All the procedures for animal surgery and pre- and post-operative care were approved by the Ethics Committee of the Second Hospital of Shanxi Medical University and followed the relevant animal protection agreements and regulations. The mini-pig model is often used in articular cartilage research and is also a common model for the study of regeneration of focal cartilage defects [12,13]. Sixteen mature female mini-pigs (18 months old) were included in this study. We selected the right hind limbs of mini-pigs as the IACLR surgery group and all the left hind limbs served as the control group. The left uninjured limbs were used as a control to compare potential side-to-side differences in systemic effects and weight bearing within the same animal.

The procedures for IACLR surgery were as follows: each mini-pig received anesthesia by intramuscular injection of 125 mg Zoletil 50 (Virbac Group France); the anesthetized mini-pig was placed in the lateral position; the right hind stifle joint was opened centrally on the patella; the patella was dislocated; and the knee was flexed to expose the ACL and the lateral surface of the lateral femoral condyle. Using an ACL reconstruction guide (Arthrex, Inc.), the guide was angled 45° to the longitudinal axis of the femur and the guide pin was inserted along the guide. A hollow drill was used to drill a tunnel of about 0.8 cm in diameter along the guide pin direction, from the lateral side to the medial side of the lateral condyle of the ACL attachment. When the tunnel was almost through, a 1-mm φ guide pin was used to drill along the periphery of the tunnel, and the tunnel was gently penetrated by the same diameter thin-walled ring osteotomy to prevent splitting of the medial femoral condyle cartilage. The tendon bone segment was pushed out of the tunnel to check the integrity of the ACL attachment point. The tendon bone segment was then pushed back into the tunnel in situ, fixed with two crossed Kirschner wires in the non-rotating state, and the tails were trimmed. The joint was closed using a standard closure after the patella reduction. The exact surgical process is shown in Fig. 1.

2.2. Tissue harvesting

Mini-pigs were euthanized and the joint fluids from the left and right hind limbs of all the animals were collected at 1, 2, 3 and 4 months after operation. The left and right hind limbs were severed from the hip joint and stored at −80 °C.