Posttraumatic knee osteoarthritis following anterior cruciate ligament injury: Potential biochemical mediators of degenerative alteration and specific biochemical markers (Review)

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Abstract. As a common injury, anterior cruciate ligament (ACL) injury is unable to heal itself naturally, which possibly increases knee instability, accelerates the risk of joint degeneration and leads to knee osteoarthritis (OA) in the ACL-injured knee. Thus, ACL reconstruction using an autograft or allograft tendon is proposed to maintain the biomechanical stability of the knee joint. However, previous studies demonstrate that surgical management of ACL reconstruction failed to abrogate the development of OA completely, indicating that biochemical disturbance is responsible for the osteoarthritic changes observed following ACL injury. Inflammatory mediators are elevated subsequent to ACL injury or rupture, inducing matrix metalloproteinase production, proteoglycan degradation, collagen destruction, chondrocyte necrosis and lubricin loss. These potential biochemical mediators may aid in the development of effective biological management to reduce the onset of future posttraumatic OA. Furthermore, during the degenerative process of cartilage, there are a number of cartilage-specific biomarkers, which play a critical step in the loss of structural and functional integrity of cartilage. The present review illustrates several specific biomarkers in the ACL-injured knee joint, which may provide effective diagnostic and prognostic tools for investigating cartilage degenerative progression and future posttraumatic OA of ACL-injured patients.

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1. Introduction

Anterior cruciate ligament (ACL) rupture is one of the most common injuries in sports activity, often accompanied by articular cartilage damage and meniscal tear (1-3). ACL deficiency can significantly alter the knee joint biomechanics (4,5), which will subsequently result in secondary damage to the cartilage with increasing time from injury (6-9). As a consequence, the articular cartilage of load-bearing joints is inexorably destroyed and the cartilage degeneration develops in an accelerated rate in the ACL-deficient knee (10-12). With regards to this, ACL reconstruction with an autograft or allograft tendon becomes a major method in maintaining the stability and normal biomechanics of the knee joint.

Restoring knee joint stability, however, was unable to decrease the incidence of posttraumatic osteoarthritis (OA) following ACL reconstruction, as previous studies have indicated that cartilage degeneration or radiographic signs of OA were detected in patients with ACL-reconstructed knees several years postoperatively (13-15). In a recent study examining 210 ACL-reconstructed patients, it was stated that 71% of the patients had radiographic knee OA and 24% showed moderate or severe radiographic knee OA postoperatively (16). Collectively, these data indicate that biomechanical instability is not the only cause for the occurrence of OA in the knee joint with ACL injury. Administered within the first month following ACL tear, intra-articular interleukin (IL)-1 receptor antagonist reduced knee pain and improved function over a 2-week interval (17). In addition to biomechanical disturbance, various biochemical alterations caused by ACL injury, such as inflammatory cytokines infiltration, matrix metalloproteinase (MMP) production, proteoglycan (PG) degradation, collagen destruction, chondrocyte death and lubricin loss, are postulated to play an important role in the development of posttraumatic OA.

2. Degenerative alteration of knee cartilage: MMP production

In the ACL-injured knee, synoviocytes, chondrocytes and other intra-articular tissues are activated to produce several inflammatory mediators, such as IL-1β, IL-6, IL-8, IL-10 and tumor necrosis factor-α (TNF-α) (18,19). IL-1 modulates the metabolic balance of cartilage in dual means, decreasing the production of the cartilage matrix components and promoting the expression of MMPs, including MMP-1, MMP-3, MMP-9, MMP-10 and MMP-13 (20-23). Recruitment of IL-1 to the IL-1 receptor activates four intracellular signaling pathways, including three mitogen-activated protein kinases (MAPKs) pathways [the extracellular signal-regulated kinase (Erk), p38 MAPK, c-Jun N-terminal kinases (JNK)] and the nuclear factor-κB (NF-κB) pathway. Activation of transcription factors, such as NF-κB (activated by Erk and p38) and activator protein-1 (activated by JNK), induces the expression of numerous MMPs (24). Thus far, 24 associated products of the MMP family have been found in mammals (25). Secreted and anchored to the cell membrane, MMPs catalytically interact with various proteins, including protein components of the extracellular matrix. In a load-induced injury model on the mature bovine cartilage, Lin *et al* (26) found that the expression of MMP-3 increased consistently in the superficial-loading cartilage zone followed by cell death. MMP-3 can activate procollagenase, which induces the degradation of cartilage matrix, consequently affecting the cartilage repair. Thus, the MMP-3 selective inhibitor may be an effective option for retarding such osteoarthritic changes (27).

3. PGs degradation

PG contains a protein core with glycosaminoglycans (GAGs) side chains. As a main component, GAG is one important specific PG biomarker. In the ACL-injured knee, infiltration of numerous inflammatory cytokines accelerates the degradation of PGs and the decrease of GAGs in the cartilage to a significant extent (28). In an ACL-injured rabbit model (29), loss of GAGs was observed in the cartilage layer for a long time period. Hattori *et al* (29) found that the average thickness of the GAG-stained area in the ACL-injured group (7.7 μm) was significantly less compared to the control group (69.4 μm), even six weeks after surgery. The apoptosis of chondrocytes has been proposed to induce GAG loss. In a similar ACL-injured rabbit model, it was also proved that apoptosis of chondrocytes lead to the decrease of GAGs in the cartilage layer of the ACL insertion following resection (30).

MMPs and aggrecanases cleave the GAGs of aggrecan at specific peptide bonds and release fragments. The proteolytic cleavage process has a destructive effect, resulting in the loss of GAGs from the matrix (31). MMPs have been reported as responsible for the C-terminal catabolic processing of aggrecan, which may accelerate the loss of matrix aggrecan in the articular cartilage (32). Cleavage of aggrecan in the interglobular domain between the N-terminal G1 and G2 globular domains is considered of greatest pathological importance, as this releases the GAG-bearing region of aggrecan from the cartilage matrix (33). Furthermore, the study by Caterson *et al* (34) showed that aggrecanase is primarily responsible for the catabolism and loss of aggrecan from the articular cartilage in the early stages of arthritic joint diseases, and MMPs begin to mediate the degradation of the small proportion of aggrecan remaining in the tissue with the occurrence of collagen catabolism at later stages. The cleavage of the Asn341-Phe342 bond in the interglobular domain can be attributed to the proteolytic activity of MMPs, while aggrecanase is responsible for the cleavage of the Glu373-Ala374 bond in the interglobular domain. In a previous study, Sondergaard *et al* (35) found that inhibition of the activation of MAPK p38, p44/42 and Src family prevented the degradation of proteolytic cartilage by blocking MMP synthesis and activity and MAPK p44/42 was essential for the degradation of aggrecan mediated by aggrecanase. Understanding of these detailed signaling pathways involved in the matrix degrading process may aid in the potential usage of corresponding inhibitors to treat the damaged cartilage.

4. Collagen destruction

Collagen, as an important structural macromolecule of cartilage, contributes to ~60% of the dry weight of articular cartilage and the type II collagen accounts for 90-95% of the articular cartilage collagen. In the early stage following ACL rupture, expression of the type II and type I collagens is elevated temporarily in the articular cartilage (36). However, with the consistent infiltration of inflammatory cytokines and activation of catabolic proteinases, cartilage specific macromolecules, such as type II collagen, are destroyed. Different from PGs, collagen loss is believed to be irreversible. Subsequently, the full thickness cartilage lesion fail to heal itself with biological repair by type II collagen, rather, they become filled with a fibrocartilage repair tissue that is deficient of the biomechanical properties. IL-1 reduces the production of type II collagen by modulating the expression of transcription factors Sp1 and Sp3 and it can also downregulate the expression of transforming growth factor-β (TGF-β) type II receptor, which has a significant influence on the synthesis of type II collagen (37). In the study by Chadjichristos *et al* (38), it was found that the production of newly synthesized collagens is inhibited by IL-1β in proliferating rabbit articular chondrocytes, accompanied by a decreased level of steady-state type II collagen. IL-1β downregulates the transcription of the *COL2A1* gene through a -41/-33 basepair sequence that is bound by a multimeric complex, including Sp1 and Sp3 transcription factors. Furthermore, Porée *et al* (39) found that IL-6 and/or soluble IL-6 receptor (sIL-6R) inhibits the production of type II collagen in rabbit articular chondrocytes at the transcriptional level. IL-6, sIL-6R or a combination can reduce the Sp1/Sp3 ratio, as well as DNA-binding activities, thus inhibiting *COL2A1* transcription.

5. Chondrocyte necrosis

Several reasons were considered to be responsible for chondrocyte necrosis. One is the pullout force of ACL insertion. Previously, it was reported that ~42% of chondrocyte apoptosis and degenerative histological changes have been observed in human ACL tibial insertion cartilage from days 19 to 206 after ACL rupture (40). Due to an imbalance between cell death and

cell proliferation in the ACL insertions following ACL rupture, the decrease of chondrocytes may lead to histological changes of the cartilage layer in the insertions (41). Another reason is the large external force that results in bone marrow edema lesions (BMELs) (42). Bone marrow necrosis and fibrosis appeared in the BMELs (43). The cartilage overlying BMELs sustains irreversible injury and cartilage degeneration lasts with increasing chondrocyte necrosis even subsequent to ACL reconstruction (44). Furthermore, the abnormal biomechanics of the injured knee also influenced cartilage morphology, and posttraumatic and new bone marrow lesions (45). Articular cartilage overlying the bone bruise lesions showed chondrocytes necrosis and PGs loss (46-48).

6. Biomarkers of cartilage degeneration

ACL injury is believed to accelerate the development of knee OA. As mentioned previously, inflammatory factors continue to influence and damage the knee cartilage matrix, although the biomechanical stability recovers following ACL reconstruction. In order to effectively prevent and abrogate the degenerative alteration of knee cartilage, there is an increasing interest to improve the understanding of the early biological changes subsequent to ACL injury and at the follow-up visit for reconstructive surgery by investigating specific biomarkers in synovial fluid, serum or urine. Catterall *et al* (28) examined 21 biomarkers in serum and synovial fluid samples (including 20 in synovial fluid and 13 in serum, with 12 biomarkers measured in the two fluids). These biomarkers can be divided into PG, collagen and synovial fluid biomarkers. With the significant release of GAGs into the synovial fluid, there would be a large amount of GAGs detected in the synovial fluid (28). GAGs in synovial fluid can be one specific biomarker providing effective diagnostic information for cartilage PG turnover in the ACL-injured knee (49,50).

Within the early time following ACL injury, the collagen-related biomarkers can be detected in synovial fluid. The progression of knee OA is associated with alterations in the systemic levels of the type II collagen metabolism biological markers (51). In general, collagen biomarkers contain C-terminal cross-linked telopeptide type II collagen (CTX-II), C-terminal cross-linked telopeptide type I collagen and N-terminal telopeptides of type I collagen. Presently, there is an increasing interest in monitoring the CTX-II level for OA progression, as high levels of the cartilage turnover biomarker CTX-II predict an increased risk of radiological progression (52-54). Lohmander *et al* (52) reported the release of CTX-II in synovial fluid from patients with ACL injury. The concentrations of CTX-II in synovial fluid were much higher in patients with ACL injury compared to the healthy control group, and the mean levels of CTX-II in synovial fluid were higher compared to the healthy levels at all time intervals following joint injury.

In synovial fluid, lubricin is believed to be one specific biomarker. Lubricin is expressed by the proteoglycan 4 gene and synthesized in a number of tissues, including cartilage, meniscus and tendon (55-57). As a disease-regulating cytoprotective glycoprotein, it plays an important role in the protection of articular cartilage structure, maintenance of articular low-friction and prevention of joint OA (58-61). A clear loss of lubricin fails to present an efficient lubricating surface for the articular cartilage contact and subsequently predisposes articular cartilage surfaces to wear-induced damage. Biomechanical stimulation can modulate lubricin expression (62,63). In the ACL-injured knee, expression of lubricin becomes abnormal with the abnormal articular cartilage contact and pressure distribution (64,65), which may influence integrative cartilage repair (66). Additionally, a number of cytokines and growth factors are involved in the regulation of lubricin expression in the ACL-injured knee. As an anabolic factor, TGF-β1 upregulates the synthesis and surface localization of lubricin (67,68), whereas IL-1 or TNF downregulate the production of lubricin (69,70). In an animal model of posttraumatic arthritis, it has been demonstrated that blocking TNF increases lubricin production on the surface of articular cartilage (71). As stated, a severe inflammatory reaction will occur in the ACL-injured knee joint, followed by infiltration of various inflammatory cytokines, such as IL-1 and TNF. Consequently, the normal synthesis of lubricin is affected and lubricin production is significantly reduced with the degenerative processing of cartilage. Therefore, lubricin can be one specific biomarker in synovial fluid.

7. Conclusions and perspectives

Biomechanical instability does not appear to be the only cause for the OA changes of the knee joint following the ACL rupture and an alternative etiology by various biochemical factors are postulated to play an important role in the development of posttraumatic OA following ACL injury. In the present study, several important biological factors have been summarized, which participate in the process of articular cartilage damage. Among these factors, inflammatory cytokines were reported to play a significant role in the production of MMPs, which contribute to cartilage PG degradation and collagen destruction. A detailed understanding of these biochemical mediators involved in the matrix degrading process will aid the potential use of specific inhibitors to slow the cartilage degenerative progression and treat the damaged cartilage. Furthermore, several specific biomarkers, including GAGs, CTX-II and lubricin were reviewed. These specific biomarkers can provide effective diagnostic and prognostic tools for identifying subjects with a high risk of progression. Collectively, these results may prompt further studies on the biological prognosis and management of knee joint cartilage damage following ACL rupture.

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