Knee osteoarthritis - a pathological basis for use of newer drug therapies

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INTRODUCTION

In a young and healthy knee joint, the protective mechanisms (good muscle action, adequate nervous system control, strong ligaments, adequate joint lubrication, and bone support) cause physiological distribution of weight during movements. A pathological cycle of events in the joint may be initiated by damage due to injury, chronic overuse, mechanical strain on the joints secondary to muscle weakness or age related degeneration. Knee osteoarthritis (OA) is a disease of the “whole joint,” which involves a series of molecular changes in the cartilage and subchondral bone, which are complicated by an imbalance between the tissues (synovium, ligaments, and muscles) that make up the joint. The interaction between inflammatory, hypoxic and mechanical pathways makes the joint prone to OA.

OA - an inflammatory response

Inflammation which was earlier considered a secondary event has also shown to be a primary event in OA cartilage. Magnetic resonance imaging has demonstrated synovitis in early OA, even in the absence of clinical synovitis. The synovial tissue from patients with early OA on staining shows mononuclear cell infiltration, and the production of proinflammatory cytokines and mediators of joint damage.

OA - an autoimmune response

Risk factors induced initial injury to the cartilage results in the release of several cartilage specific auto-antigens. The joint tissues are later infiltrated by T-cells, B-cells, and macrophages. Anti-bodies have been detected in patients with early-stage knee OA indicating an autoimmune response, but not in those with late-stage knee OA. Circulating systemic markers of inflammation, such as C-reactive protein (CRP), may be elevated in serum of OA patients compared with their controls. Elevated levels of CRP have been correlated with the degree of synovial fluid infiltration as well as symptoms of pain and stiffness. A search of literature was done using Google, PubMed, Cochrane databases, and Science Direct for the last
7 years (2005-2012). Key words such as OA, pathogenesis, newer drugs, and pathological targets were used.

**PATHOLOGICAL CHANGES IN CARTILAGE**

The two main constituents of the cartilaginous extracellular matrix (ECM) are a Type II collagen-rich collagenous network, which provides tensile strength, and a proteoglycan called aggrecan, which allows cartilage to resist a compressive load. The ECM is constantly remodeled through degradation followed by synthesis to maintain the cartilage structure. In OA, the degeneration of the ECM is much more than its synthesis. Hence, the ECM of cartilage wears away, exposing articular cartilage and later on, the bone. The various factors that are responsible for pathological changes in the joint are cytokines (interleukins [ILs], tumor necrosis factors [TNFs], insulin-like growth factors [IGFs], transforming growth factors [TGFs]), metalloproteinases (MMPs), prostaglandins (PGs), and oxidative stress and transcription factors.

**Cytokines**

Cytokines are polypeptide products produced mainly during immune and inflammatory processes that are released from one cell type and modulate the function of other cell types. Cytokines involved in OA are released from chondrocytes, synovial cells, or osteocytes.

Cytokines involved in cartilage metabolism are divided into three categories:

- **Catabolic cytokines** (IL-1β, TNFα, IL-17, and IL-18),
- **Inhibitory cytokines** (IL-4, IL-10, IL-11, IL-13, IL-1 receptor antagonist, and interferon-γ) and,
- **Anabolic cytokines** (IGF-1, TGF-β1, TGF-β2, TGF-β3, fibroblast growth factors [FGF-2, FGF-4, FGF-8], bone morphogenetic proteins [BMP-2, BMP-4, BMP-6, BMP-7, BMP-9, and BMP-13]).

**IL-1 and TNFα**

IL-1 and TNFα are the most well studied cytokines in OA. TNFα and IL-1 can inhibit the synthesis of proteoglycans and Type II collagen. They can significantly up-regulate MMP gene expression. IL-1 induces the synthesis of prostaglandin E2 (PGE2) and the production of nitric oxide (NO) through inducible NO synthetase (iNOS, or NOS2). IL-1β also induces IL-6, leukemia inhibitory factor, IL-17, and IL-18 and chemokines.

**IGFs**

IGF-1 (a structural and functional analog of insulin) promotes chondrocyte proliferation and differentiation but inhibits apoptosis. Insulin-like growth factor binding proteins (IGFBPs) are a group of secreted proteins, which bind to IGF-1 and modulate its biological actions. The articular cartilage and synovial fluid from patients with OA revealed increased IGFBP levels. IGFs-independent signals for chondrocyte survival are delivered by over-expression of IGFBPs.

**TGF-β**

TGF-β regulates cellular proliferation, differentiation, and ECM function. Its isoforms signal through a pair of transmembrane serine/threonine kinases. The deregulation of its signaling has been implicated in OA. TGF-β stimulates collagen and proteoglycan synthesis and reduces the activity of IL-1β-stimulated MMPs. BMP-2 produced by macrophages promotes osteophyte formation by enhancing chondrogenesis and osteogenesis. In articular chondrocytes, isolated from knee joints from patients with OA pre-treatment with IL-1β was shown to reduce TGF-β-induced activity.

Cytokine induced cartilage degradation is mediated by MMPs, including a disintegrin and MMP with thrombospondin motifs (ADAMTS).

**MMPs**

MMPs belong to a huge family of enzymes that degrade different components of collagen and proteoglycans. MMPs are divided into five groups namely collagenase, stromelysin, gelatinase, membrane type MMPs, and others. MMP-1, MMP-3, MMP-2, and MMP-9, MMP-8, MMP-13, and aggrecanase have been well-studied experimentally. Tissue inhibitors of MMPs (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) regulate MMP activity by inhibiting them. If TIMPs do not inhibit MMPs, they will degrade both the endogenous and newly synthesized ECM proteins. MMP-1 and MMP-13 are rate limiting in the process of collagen degradation. MMP-13 degrades both collagen and aggrecan. Aggrecanases (ADAMTS-4/-5) specifically cleave the aggrecan molecule in a particular region thereby destroying its activity.

**PGs**

PGs influence the sensitivity of spinal cord neurons and thereby contribute to pain hypersensitivity. PGE2 can also inhibit growth plate chondrocyte differentiation by gene down-regulation. Low concentrations of this PG are capable of increasing chondrocyte proliferation. Overproduction can enhance NO-induced cell death of OA chondrocytes.

**Subchondral bone changes**

Bone remodeling and attrition occur relatively early in the disease process. Use of fractal signature analysis showed that bone loss occurred in patients with knee
OA and that changes were associated with an increase in the number and size of the remodeling units.38 The subchondral bone contributes to OA due to a defect in its role as a shock absorber; abnormal osteocyte function or increased production of bone-derived products, cytokines, and MMPs.38,39 Bone marrow lesions have been found to be more common in persons with painful knees compared with persons with no knee pain and are related to pain severity.40

**Angiogenesis**

Angiogenesis and inflammation are closely integrated processes in OA. Angiogenesis may promote chondrocyte hypertrophy and endochondral ossification. Along with inflammation, it may sensitize nerves and thus increase pain. Innervation may follow vascularization of the articular cartilage and these nerves can in turn be stimulated by compressive forces and hypoxia.41 The endogenous angiogenesis inhibitors and matrix constituents, as well as growth factors produced by chondrocytes, subchondral bone and synovium regulate the blood vessel growth in cartilage.42 Blood vessels from the subchondral bone invade the articular cartilage facilitating the progression of OA and forming osteophytes in the process.43 Synovial neovascularization takes place secondary to synovitis as macrophages in themselves secrete angiogenic factors such as vascular endothelial growth factor (VEGF), and can stimulate other cells to secrete angiogenic factors.41 Anti-angiogenic factors like Angiopoietin (Ang)-2 which cause vascular regression are also up-regulated during synovitis. Ang-2 also facilitates inflammation.44 Vascular immaturity and redistribution of blood vessels away from the synovial surface may deprive the articular cartilage of its nutrition.45

Hypoxia also plays a role in angiogenesis. Up-regulation of hypoxia inducible factor-1α in the osteoarthritic synovium is also associated with increased microvascular density and expression of angiogenic factors.46 VEGF may facilitate the production of MMPs, especially under hypoxic conditions.47

**Oxidative stress**

Articular cartilage is an avascular tissue, and hence oxygen supply is reduced. Chondrocytes are oxygen sensitive. Besides the influence of oxygen itself, reactive oxygen species (ROS) including hydroxyl radical, superoxide anion and hypochlorite ion, as well as O$_2^-$-derived non-radical species, such as hydrogen peroxide play a crucial role in the regulation of a chondrocyte activities such as cell activation, proliferation and matrix remodeling. When ROS production exceeds the antioxidant capacities of the cell, oxidative stress produced will lead to cartilage damage.48 ROS are responsible for matrix and cartilage degeneration and apoptosis of chondrocytes.49,50 The serum of the OA patients has a low concentration of antioxidant activity markers and higher antioxidant concentration could mean better the cartilage metabolism.49

**Transcription factors**

Down-regulation of transcription factors like SOX9 in the hypertrophic zone of the normal growth plate is essential for allowing vascular invasion, bone marrow formation and endochondral ossification.51

All the above-mentioned pathological changes are together responsible for OA symptoms and progression. Hence, all the newer drug therapies for OA are directed toward modifying these pathological targets in the hope to prevent progression of OA.

**DRUG THERAPIES**

The pharmacological treatment includes two major groups:

a. Fast-acting agents: analgesics, non-steroidal anti-inflammatory drugs and corticosteroids

b. Slow-acting drugs
   - Cartilage matrix precursors: glucosamine, chondroitin sulfate, hyaluronic acid (HA) and vitamins/minerals
   - Modulators of cytokines: diacerein, MMP inhibitors, etc.

Other supplements like sulfur/methionine containing molecules, avocado/soybean unsaponifiables (ASU), omega-3 polyunsaturated fatty acids (PUFAs), vitamins and minerals are also being routinely used.

Slow-acting drugs have a slower onset, lesser side-effects and their effects last for months after treatment discontinuation. They are prescribed as drugs in European countries and sold as nutraceuticals in USA. Even though, the fast acting agents are the mainstay of the OA management, the slow acting agents are being increasingly looked as disease modifying agents. The various pathological modifications produced by these drugs are as described:

**Glucosamine sulfate**

- Increases HA production in human synovium.52
- Inhibits aggrecanase by suppression of glycosylphosphatidylinositol-linked proteins.53
- Inhibits the expression and activity of ADAMTS-5.54
- Prevents activation of human chondrocytes by IL-1β and thereby inhibits the release of cyclooxygenase 2 (COX-2), IL-6, and NO.55
- Reduces the NO-induced cell death of chondrocytes by an antioxidant action thereby reducing iNOS expression and activity.56
- Increases the osteoprotegerin/receptor activator of nuclear factor kappa-B (NF-κB) ligand ratio and reduces bone resorption. This effect increases when glucosamine is used in combination with chondroitin sulfate.57
Chondroitin sulfate

- Increases the hyaluronan production by human synovial cells.\(^{58}\)
- Inhibits the enzymes leukocyte collagenase, elastase and hyaluronidase and inhibits collagen breakdown in chondrocytes.\(^{59}\)
- Reduces the formation of IL-1\(\beta\) and TNF-alpha and COX-2 and NOS-2.\(^{60}\)
- Exhibits an antioxidant action by reducing the NO-induced cell death of chondrocytes.\(^{60}\)

It has both anabolic effects (promotes proteoglycan production) and anti-catabolic (inhibits collagen breakdown in chondrocytes) effects on cartilage metabolism. It has also been proposed that the sulfate moiety of both chondroitin sulfate and glucosamine sulfate may contribute significantly to their in vivo activity.\(^{61}\)

Sulfur

Sulfur, in the form of sulfate, is needed to maintain the integrity and function of articular cartilage. The cartilage matrix is created by sulfating monomers along the chondroitin sulfate chain thereby serving as an effective cushion during weight bearing. The three commonly used sulfur/methionine containing molecules are S-adenosylmethionine (SAMe), dimethyl sulfoxide (DMSO), and methylsulfonylmethane (MSM, sometimes called dimethyl sulfone DMSO2).\(^{71}\)

a. SAMe: it improves proteoglycan metabolism and has a direct anti-inflammatory activity.\(^{72}\)

b. DMSO and MSM: they reduce peripheral pain and might inhibit the degenerative changes occurring in OA. They reduce inflammation by stabilizing cell membranes, slowing or stopping leakage from injured cells and scavenging hydroxyl free radicals which trigger inflammation.\(^{74}\)

HA

- Inhibits PGE2 synthesis in human OA synovial cells, and limits leukocyte adherence, proliferation, migration, and phagocytosis.\(^{62}\)
- Traps endogenous pain substances and decreases activation of joint pain fibers secondary to coating of their receptor endings with viscous HA.\(^{62}\)
- Stimulates the production of TIMP-1 by chondrocytes, inhibits neutrophil-mediated cartilage degradation and attenuates IL-1 induced matrix degeneration and chondrocyte cytotoxicity.\(^{62}\)

HA has a mild anti-inflammatory and anti-apoptotic effect.\(^{63}\)

Diacerein

- Metabolized to rhein, an agent that has anti-inflammatory and analgesic properties. Diacerein is shown as an inhibitor of production and activity of IL-1 (both in vivo and in vitro).\(^{64}\)
- Inhibits the release of inflammatory and cartilage degrading factors, by inhibiting the activation of NF-\(\kappa\)B,\(^{65}\) and stimulates the production of cartilage growth factors and cartilage components, even in the presence of IL-1\(\beta\)\(^{66,67}\).
- Inhibits superoxide production, neutrophil chemotaxis and phagocytosis and macrophage migration and phagocytosis. Diacerein reduces the IL-1\(\beta\)-induced MMP-13 production in OA subchondral bone.\(^{68,69}\)
- Reduces the synthesis of resorptive factors and osteoclast formation.\(^{69}\)

Tetracyclines

Tetracyclines are anti-biotics with an anti-inflammatory effect mediated by inactivation of cartilage MMPs.\(^{70}\)

Selenium, zinc, and copper

When low selenium diet was fed to rats, sulfotransferase enzyme activity was found to be decreased. This enzyme is
required for glycosaminoglycan synthesis.\textsuperscript{84} Zinc increases bone formation and mineralization, decreases bone resorption and stimulates collagen production.\textsuperscript{85} Manganese is required for enzymes involved in the glycosaminoglycan synthesis and the cross-linking of collagen fibrils.\textsuperscript{86} Copper is also required for enzymes like lysyl oxidase, involved in the cross-linking of collagen and elastin in cartilage and bone.\textsuperscript{87}

**Silicon and boron**

Silicon promotes bone formation and inhibits osteoclast mediated bone resorption. It stimulates DNA synthesis in osteoclast like bone forming cells. It also increases the synthesis of collagen.\textsuperscript{88} Boron appears to participate in hydroxylation reactions, which play a role in the synthesis of steroid hormones and vitamin D.\textsuperscript{89} Low boron intake results in impaired bone health and immune response.\textsuperscript{90,91}

**CONCLUSION**

This review tries to provide an overview of the pathological targets for newer therapies in OA. Studies on the mechanism of action of these drugs are based on animal and in vitro studies and hence it is difficult to actually predict their action in human cartilage. These drugs are being favored in clinical practice in various combinations because of their novel mechanisms of action and better tolerability profile. Even though these drugs appear to be disease modifying in their action, they have not been recommended by latest OA management guidelines. Long-term clinical studies are required to confirm its promising status as disease modifying agents in OA.

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