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Mitogen-Activated Protein Kinases as Therapeutic Targets in Osteoarthritis

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Abstract

Purpose of review—The mitogen-activated protein (MAP) kinases are intracellular signaling proteins which play a central role in controlling the activity of pathways that regulate production and activity of multiple mediators of joint tissue destruction. The therapeutic potential of MAP kinase inhibition in osteoarthritis was reviewed.

Recent findings—Results from basic research studies support the role of MAP kinases as central mediators that regulate expression of pro-inflammatory cytokines and metalloproteinases but also as potential pain mediators as well. Cell culture and animal model studies suggest that inhibition of MAP kinases might slow progression of osteoarthritis but trials of MAP kinase inhibitors in humans with osteoarthritis have not yet been reported. Safety concerns of the currently available inhibitors have limited their initial use to trials in conditions considered more severe than OA.

Summary—MAP kinase inhibition has the potential to slow disease progression in osteoarthritis and also might reduce pain; however, safety concerns have limited the use of general MAP kinase inhibitors in humans. Further understanding of the function of specific isoforms of the MAP kinases as well as upstream and downstream effectors may lead to the development of more specific inhibitors with less toxicity that could eventually be used as structure-modifying drugs for OA.

Keywords

Osteoarthritis therapy; articular cartilage; MAP kinases

Introduction

Progression of osteoarthritis (OA) is characterized by destruction of the articular cartilage and other soft tissues in the joint, such as ligaments and, in the knee, the menisci. This tissue destruction is accompanied by remodeling and hypertrophy of neighboring bone and varying degrees of synovial inflammation [1*]. The destruction, remodeling, and inflammation of joint tissues leads to joint failure manifested as joint pain and loss of function. New treatments for OA, sometimes called structure-modifying OA drugs (SMOADs), are needed that can slow or halt the structural changes in joint tissue with the anticipation that this will result in significant pain reduction and improvement in function.

The structural changes in an OA joint are thought to be due to an imbalance in degradative (catabolic) and synthetic (anabolic) activity resulting in excessive production of matrix degrading enzymes and insufficient matrix repair. Contributing factors to the imbalance in

homeostasis include the direct effects of mechanical loading on joint tissues, autocrine and paracrine signaling initiated by cytokines and the damaged matrix itself (matrix fragments), and an alteration in the phenotype of the chondrocytes which are the cells in the articular cartilage responsible for maintenance of the cartilage matrix [1*-3**]. Inhibiting the cell signaling pathways that regulate catabolic and anabolic activity would theoretically restore homeostasis in the joint and perhaps promote repair.

Central to the matrix damage that occurs in OA is the increased production, by chondrocytes, of matrix degrading enzymes including the matrix metalloproteinases (MMPs). Inhibition of the synthesis and/or activity of MMPs has been a focus in developing structural-modifying treatments for OA. However broad-spectrum MMP inhibitors have not been shown to slow progression of OA and appear to have dose limiting toxicity due to musculoskeletal side effects [4]. Inhibitors with greater specificity for MMPs thought to be central to OA, such as MMP-13 and aggrecanase-2, are being developed which might have greater efficacy and less toxicity. As an alternative to directly inhibiting MMP activity, the signaling pathways involved in MMP regulation are also being explored as therapeutic targets. MMP production is regulated by several signal transduction pathways, including those of the mitogen-activated protein (MAP) kinases. Because the MAP kinases are also central regulators of additional cell signaling pathways that control cell proliferation, survival, matrix synthesis, and production of pain mediators, they have been considered to be potential therapeutic targets for several diseases including osteoarthritis.

MAP kinase signaling

The MAP kinases are Ser/Thr kinases divided into 4 subfamilies that include extracellular regulated kinase (two isoforms - ERK 1 and 2), c-Jun NH₂-terminal kinase, also called stress activated protein kinases or SAPK (three isoforms - JNK 1,2, and 3), p38 (four isoforms - α , β , γ , δ), and ERK5 [5]. Less is known about ERK5 and it has not been studied in relation to arthritis so this review will focus on the others. The MAP kinases are activated by a diverse range of stimuli including cytokines, growth factors, and matrix proteins that bind to various receptor tyrosine kinases, G-protein coupled receptors, cytokine receptors, and integrins. Signals generated from these cell surface receptors initiate a cascade of signaling events that lead downstream to the activation of one or more of the MAP kinases (Figure 1). The involvement of multiple signaling proteins within a complex signaling network allows for regulation at various steps in the pathway and integration of signals from a diversity of stimuli.

The MAP kinases are activated by phosphorylation of specific Thr and Tyr residues by MAP kinase kinases (MKKs). ERK1 and ERK2, which are both widely expressed in multiple cell types including various joint tissues and are most commonly found together, are activated by MKK1 and MKK2 respectively, which are also referred to as MAP and ERK kinases or MEKs 1 and 2 [5]. Classic inhibitors used in many *in vitro* studies of ERK1/2 are PD98059 and U0126 which inhibit the activity of MEK1/2. p38 is also widely expressed although most tissues do not express all 4 isoforms. MKK3 and MKK6 are the most common activators of p38 and p38 is inhibited directly by several compounds including SB203580 which inhibits p38 α and p38 β , but not p38 δ or p38 γ [6]. JNK1 and JNK2 are widely expressed in tissues, while JNK3 expression is limited to mainly brain, testes, and heart tissue [7]. JNK is most often activated by MKK4 and MKK7 and can be inhibited by compounds such as SP600125 which inhibits all 3 isoforms [8]. Recent work in synoviocytes from patients with rheumatoid arthritis has shown that IL-1 activation of JNK by MKK4 and MKK7 requires the upstream activity of the TGF- β activated kinase 1 (TAK1) [9].

Once activated, the MAP kinases in turn, activate other protein kinases and several transcriptional regulatory proteins [5]. The latter include Elk-1, Ets, c-Myc, c-Jun, ATF-2, p53

and MEF2. Probably best characterized is ERK activation of Elk-1 and c-Myc, p38 activation of ATF-2 and MEF2, and JNK activation of c-Jun. These transcription factors regulate the expression of a host of genes relevant to OA including genes involved in the inflammatory response, regulation of cell proliferation, and production of matrix degrading enzymes such as MMPs. JNK may be particularly important because of its unique ability to activate c-jun, a key AP-1 component [7]. Besides promoting MMP expression, AP-1 can regulate the expression of pro-inflammatory cytokines such as TNF α and IL-1. These cytokines can then act in an autocrine/paracrine manner to maintain JNK activation and activate JNK in additional cells, further increasing cytokine and MMP production. In this way, JNK integrates many signals generated by different stimuli to amplify the normal levels of MMP production, making it a potential target for treating OA.

MAP kinase function with relevance to osteoarthritis

MAP kinases have been shown to be activated in OA cartilage and there is evidence, at least for ERK, that they can play a key role in the cartilage destruction seen in OA. Levels of activated (phosphorylated) JNK in human OA cartilage appear to be greater than the levels present in normal cartilage [10,11]. Phosphorylation of p38 was also higher in human OA compared to normal tissue while phosphorylated ERK was found in both [11]. In a dog model of surgically-induced OA, ERK1/2, JNK, and p38 were all activated to a greater degree in OA compared to normal tissue [12]. However, results of immunohistochemistry experiments examining MAP kinase phosphorylation *in situ* must be interpreted with caution because of problems with antibody specificity, the transient nature of phosphorylation, and the possibility that alterations in phosphorylation may occur during tissue processing.

In the dog OA model, a compound (PD-0200347) that serves as a ligand of voltage gated Ca⁺⁺ channels reduced the levels of phosphorylated ERK, but not p38 or JNK [12]. This compound had been previously noted to reduce the development of OA lesions in the dog model in association with a reduction in MMP production [13] suggesting that inhibition of ERK activation was sufficient to reduce OA lesions in this model. In support of this, a study from the same group using a rabbit model of OA demonstrated that a MEK 1/2 inhibitor, which blocks ERK activation, also reduced the severity of the OA lesions [14]. Likewise, avocado-soybean unsaponifiables (ASU), which are used to treat OA in Europe, were reported to inhibit IL-1 induced ERK but not p38 or JNK in chondrocytes *in vitro* [15]. Whether JNK or p38 specific inhibitors would give similar results to ERK inhibition has not been determined.

Recent studies have continued to support a key role for MAP kinases in the regulation of MMP production by chondrocytes. In addition to cytokine regulation of MMP expression [11], MAP kinases are required for induction of MMPs by matrix fragments generated during the development of arthritis including fibronectin fragments [16] and collagen fragments [17*, 18*]. MAP kinases, at least p38, also play a role in MMP expression induced when type II collagen binds to the discoidin domain receptor. Inhibition of any one of the 3 major MAP kinases (ERK, p38, and JNK) was able to inhibit fibronectin fragment induced MMP-13 expression [16] while only p38 inhibition, and not JNK, blocked MMP-13 expression mediated by the discoidin domain receptor [17*]. Similar to fibronectin fragments, stimulation of MMP-13 by bFGF required activity of ERK, p38, and JNK [19,20].

Cytokine stimulation of MAP kinase activity may also contribute to the development of OA through down regulation of peroxisome proliferators-activated receptor gamma (PPAR γ). Activation of PPAR γ has been shown to have chondroprotective effects in OA associated with reduced activation of ERK1/2 and p38 [12]. But OA cells appear to express less PPAR γ than normal chondrocytes, possibly due to the downregulation of PPAR γ by IL-1 [21]. In this study,

inhibition of either p38 or JNK, but not ERK, was found to prevent IL-1 induced down regulation of PPAR γ in chondrocytes.

MAP kinases are also involved in the regulation of chondrocyte anabolic activity. IGF-I stimulation of proteoglycan synthesis requires activity of the PI-3 kinase, while there is some evidence that ERK may be a negative regulator of proteoglycan synthesis [22]. A recent study found that chondrocytes express receptors for sphingosine-1-phosphate, a metabolite of ceramide, and that sphingosine-1-phosphate stimulation of ERK and p38 was associated with inhibition of aggrecan expression [23]. Treatment with the MAP kinase inhibitors PD98059 and SB203580, to inhibit ERK and p38 α respectively, blocked sphingosine-1-phosphate induction of PGE₂, but the effect of these inhibitors on aggrecan expression was not determined. LPS activation of toll-like receptor 4 can inhibit chondrocyte proteoglycan synthesis and under these conditions inhibition of p38, but not ERK, partially restored it [24]. In contrast to the inhibition of proteoglycan synthesis by p38 and ERK, a JNK-dependent increase in proteoglycan synthesis was demonstrated in chondrocyte cell lines following cyclical mechanical stimulation [25].

The role of the MAP kinases in OA tissues other than the articular cartilage has received little attention. A recent study found evidence for p38 activation in ruptured menisci [26]. Another recent report found that MMP-1 production by OA synovial fibroblasts stimulated with adiponectin required p38 activity [27]. Other stimuli that may contribute to the development of OA in older adults include basic calcium phosphate crystals that are commonly found in OA joints. These crystals can stimulate MMP production by chondrocytes or by synovial fibroblasts. Recent work in fibroblasts demonstrated that activation of ERK was required for basic calcium phosphate crystals to stimulate c-fos which is a regulator of MMP expression in these cells [28]. Another recent study has shown a role for both ERK and p38 in the calcium phosphate crystal induced MMP-13 production by OA synovial fibroblasts [29]. In addition, p38 has been shown to mediate increased chondrocyte MMP-3 expression in response to monosodium urate crystals [30].

Besides a role in regulating processes relevant to joint tissue destruction, mounting evidence suggests that MAP kinases may also be important in mediating pain signals. In an animal model of inflammatory joint pain, rats were injected with Freund's adjuvant in the knee joint to induce inflammation and this was found to induce activation of ERK in dorsal root ganglions [31*]. In this study, intrathecal injection of the MEK inhibitor UO126 was found to reduce pain behavior elicited by passive motion of the inflamed joint, suggesting a role for ERK signaling in joint pain. Studies outside of the joint have also suggested that ERK may have a role in pain signaling [32,33]. In addition to ERK, there is evidence that p38 may also play a role in neuropathic pain and a clinical trial of a p38 inhibitor for the treatment of neuropathic pain is listed on www.clinicaltrials.gov.

Inhibition of MAP kinases: potential benefit versus risks

Despite initial animal model studies suggesting that MEK/ERK inhibition would be a potential therapeutic target in OA [14], inhibitors of the ERK pathway have received little attention in additional animal model studies or in clinical trials in humans. This is most likely because of the potential toxicity of systemic ERK inhibition given the knowledge that the ERK MAP kinase pathway is involved in a multitude of growth factor signaling pathways that regulate cell proliferation and tissue homeostasis. JNK and p38 are most commonly activated by inflammatory and stress-induced signals and so inhibition of these MAP kinases is thought to have a better risk-benefit ratio than ERK inhibition.

A number of JNK inhibitors, both direct and indirect, have been developed and tested as potential therapeutics for several diseases, including rheumatoid arthritis (RA), which like OA

results in joint destruction despite its different etiology. In rat adjuvant arthritis, a model for inflammatory arthritis, subcutaneous administration of the JNK inhibitor SP600125 significantly reduced joint damage (cartilage and bone erosion) in comparison to the vehicle control, while only a modest decrease in paw swelling/inflammation was observed [8]. SP600125 treatment also markedly decreased JNK kinase activity, AP-1 activation, and MMP-13 mRNA levels in the synovium [8]. Treatment of TNF α -stimulated synoviocytes with R406, a novel inhibitor of spleen tyrosine kinase (Syk), markedly suppressed JNK activation, AP-1 DNA binding, and MMP expression, while having minor effects on p38 and ERK [34]. Celecoxib, one of the commercially available selective COX-2 inhibitors, was also shown to inhibit IL-1 β -stimulated JNK and NF κ B activation (but not p38 or ERK), resulting in a significant suppression of MMP production in normal, OA, and RA articular chondrocytes [35].

Although JNK inhibitors have not been tested in animal models of OA, there is evidence indicating JNK inhibitors may effectively prevent further cartilage degradation in OA. Diverse stimuli found to be present in OA synovial fluid and cartilage, including TNF α , IL-1, fibronectin fragments, and bFGF all require JNK activation for stimulation of chondrocyte MMP production, suggesting it is a key mediator of MMP production in articular cartilage [20,36-38]. In the rat adjuvant arthritis study by Han et al [8], the finding that cartilage erosion was reduced to a greater degree than joint inflammation suggests that JNK inhibition might have effects on the cartilage independent of the synovium. Although the synovial inflammatory response is likely the primary initiator of joint damage in RA, it is believed that OA synovial inflammation is secondary to the initial cartilage damage. Thus, inhibition of JNK may be more effective in the treatment of OA in comparison to its use as an RA therapeutic.

Enthusiasm for the use of JNK inhibitors in the treatment of OA has been dampened by concerns about toxicity. The JNK inhibitors recently examined in clinical trials listed on www.clinicaltrials.gov for early Parkinson's disease and myeloid leukemia were found to be ineffective at the doses tolerated leading to the studies' termination. Possible problems with inhibiting JNK may be due to the important role it plays in other cellular functions such as cell proliferation, cell differentiation, the inflammatory response, and apoptosis. JNK is required for both T-cell function and the regulation of non-transcription factors like Bcl-2 family members that are involved in programmed cell death [7]. Until new and improved inhibitors are developed, the side effects and toxicity typically observed with the currently available JNK inhibitors may outweigh the benefits of using them for the treatment of OA.

p38 inhibition has been suggested to be potentially beneficial as therapeutic strategy in inflammatory disease processes such as sepsis, Crohn's disease, and rheumatoid arthritis [6]. This is mainly due to the ability of p38 to control inflammatory cytokine expression. Several different p38 inhibitors have been tested in animal models of rheumatoid arthritis [39-41]. In each of these studies, p38 inhibition was shown to reduce disease severity and maintain joint integrity with a reduction in the loss of cartilage and bone.

Several p38 inhibitors have advanced into clinical trials in human subjects for treatment of rheumatoid arthritis, but only a few have made it as far as phase II. The problem with these compounds has been similar to that seen with the JNK inhibitors with a poor safety profile, including adverse effects in the central nervous system and liver [42]. Due to these safety issues, new strategies for p38 inhibition are under consideration. One strategy is to investigate molecules downstream of p38 that may be more selective in their biological activities [42]. In fact, a recent study using an animal model of rheumatoid arthritis (collagen-induced arthritis) has shown that MAPKAP kinase 2 (MK2), a molecule downstream of p38, is important in disease progression [43]. It remains to be seen if this molecule or other molecules downstream of p38 could be a therapeutic target in arthritis. A second potential strategy is to investigate

the role of p38 isoforms other than p38 α [6]. Most studies to date have focused almost exclusively on p38 α and inhibitors that target this particular isoform. Not as much is known about the role of the other isoforms in the context of arthritis, although a recent report has been published demonstrating the activation of both p38 α and p38 γ within synovial tissue of rheumatoid arthritis subjects, suggesting that both of these molecules may be important in the disease process [44].

Conclusion

Accumulating evidence supports a central regulatory role of the MAP kinases in mediating inflammatory and matrix degrading processes that contribute to joint tissue destruction in osteoarthritis. Because MAP kinases also appear to be involved in pain signaling pathways, the potential exists for MAP kinase inhibitors to reduce both pain and structural progression in OA. However, due to the toxicity of the currently available MAP kinase inhibitors, risk-benefit considerations have precluded their use in clinical trials in humans with OA. New strategies are emerging such as the development of non-ATP competitive and isoform specific inhibitors that may have greater specificity with reduced toxicity [42,45]. An alternative is to inhibit proteins downstream of the MAP kinases that mediate the effects of MAP kinase activation in OA. Further work is needed to better identify the details of the MAP kinase pathways involved in OA, including tissues other than articular cartilage.

Acknowledgments

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Figure 1. MAP kinase signaling pathways

A diverse array of receptors, including growth factor, cytokine, and integrin receptors, activate signaling pathways which lead to the activation of the MAP kinase pathways. MAP kinase kinase kinases (MAPKKKs) activate MAP kinase kinases (MKKs; MAP and ERK kinase or MEK), which in turn activate the MAP kinases (p38, ERK1/2, and JNK). The MAP kinases activate other kinases, such as p90RSK, and transcription factors that regulate gene transcription including Elk-1, MEF2, ATF2, c-Myc, Ets-1, and c-Jun. In this way, all three MAP kinases are involved in the expression of matrix metalloproteinases (MMPs) and other mediators involved in the development of OA.