2. Cyclooxygenase-2 and microsomal prostaglandin E synthase-1 in the pathophysiology of osteoarthritis

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Abstract. The exact etiology of osteoarthritis (OA) is not fully understood and as a result no appropriate curative therapeutics are currently available to halt the progression of this disease. Mediators derived from the arachidonic acid (AA) metabolic pathway, especially prostaglandins (PGs), are believed to play a crucial role in the pathophysiology of this disease. Among the various PGs, PGE₂ is the major AA metabolic product involved in inflammatory and destructive mechanisms associated with OA. Two key enzymes involved in the biosynthesis of PGE₂ are cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1). Non-steroidal antiinflammatory drugs, which act via COX inhibition, resulting in subsequent inhibition of PGE₂, are extensively used for the management of inflammation, pain, swelling and joint stiffness associated with OA. This chapter will summarize the current knowledge of the role of COX-2 in OA. In addition, the emerging role of mPGES-1 as a potential therapeutic target for OA will be discussed.
Introduction

Arachidonic acid (AA) is an essential fatty acid which is bound to membrane phospholipid molecules, triglycerides and cholesterol esters. During an event of tissue injury, stress or cell damage, cell membrane phospholipids activate phospholipases to liberate AA which can be further metabolized by at least three different enzymatic systems. One of the most critical enzymes in the AA metabolic pathway is cyclooxygenase (COX), which metabolizes AA into prostaglandin (PG)G_2 by its COX activity and then into the intermediate substrate PGH_2 by its peroxidase activity. PGH_2 is further metabolized by individual PG synthases into PGE_2, TXA_2, PGD_2, PGF_{2α} and PGI_2 [1] (Figure 1). AA can also be metabolized by the lipoxygenase (LOX) enzymes to generate leukotrienes and hydroxyeicosatetraenoic acids. In addition, recent studies have demonstrated that lipoxins are generated by a unique AA metabolic pathway mediated by both COX and LOX [2].

Figure 1. Biosynthesis of prostaglandins and thromboxanes.
Cyclooxygenases

In 1971, Sir John Robert Vane first demonstrated that the mechanism of action of aspirin and other non-steroidal antiinflammatory drugs (NSAIDs) occurs via the inhibition of PG production [3]. Subsequently, two COX isozymes were identified, namely COX-1 and COX-2 [4-6]. COX-1 is a 69-kd protein, its gene resembles a housekeeping gene and lacks a TATA box [7] and two Sp1 cis-regulatory elements, contributing to its constitutive expression [8]. COX-2, on the other hand, is a 72-kd protein and its promoter contains a TATA box and several inducible enhancer elements, most notably the nuclear factor-κB (NF-κB), cyclic adenosine monophosphate response element and the CCAAT enhancer binding protein.

COX-1, which is constitutively expressed in the majority of mammalian cells and tissues, regulates the production of PGs and thromboxanes (TXs) involved in the regulation of vascular, gastrointestinal and renal homeostasis [9]. In contrast, COX-2, which is induced in response to a variety of proinflammatory stimuli, regulates the production of PGs involved in inflammation, pain, and fever, especially PGE$_2$ and prostacyclin (PGI$_2$) [10-13]. COX-3 was reported to be a novel COX isozyme predominantly expressed in the cerebral cortex and heart [14]; however, its existence in humans has been questioned by a subsequent study [15].

Cyclooxygenases in articular cells and tissues

COX-2 is not expressed in unstimulated normal human articular chondrocytes, but is induced by inflammatory mediators including interleukin (IL)-1β, IL-17, tumour necrosis factor α (TNF-α), leukemia inhibitory factor and bacterial lipopolysaccharide [16-18]. It is expressed in high levels in cartilage from OA patients [17, 19, 20] and its increased expression is associated with the release of PGE$_2$. Since the production of proinflammatory PGs, especially PGE$_2$, at sites of inflammation coincides with the upregulation of COX-2 expression in activated articular cells, COX-2 has long been a key target for the treatment of osteoarthritis (OA) and other forms of arthritis.

The significance of COX-2 in arthritis has also been demonstrated in various experimental models involving cartilage destruction. A selective COX-2 inhibitor significantly inhibited the pathophysiological symptoms including paw edema, spontaneous pain, and hyperalgesia in an adjuvant induced arthritis (AIA) rat model [21]. However, in another study in the same model, a COX-1 selective inhibitor did not reduce the inflammation or PGE$_2$ production [22]. In addition, a selective COX-2 inhibitor, but not a selective
COX-1 inhibitor, reduced the severity of symptoms in a type II collagen-induced arthritis (CIA) mouse model [23]. Furthermore, COX-2-deficient mice, but not COX-1-deficient mice, displayed a significant reduction in both clinical and histological signs of CIA [24].

COX-2 expression is mediated by several signalling pathways depending on the type of tissue/cell and on the stimulus. In chondrocytes and synovial fibroblasts, some of the most important signalling pathways involved in mediating COX-2 expression include the mitogen-activated protein kinase signalling cascades JNK/SAPK and p38 [25]. β-catenin has also been shown to regulate the expression of COX-2 in articular chondrocytes [26]. NF-κB is another key mediator involved in the regulation of COX-2 in articular cells [27]. The latter appears important as the majority of COX-2-inducing mediators also activate NF-κB, and the COX-2 promoter contains 2 consensus NF-κB binding sites [27-29].

Prostaglandin E synthases

To date, various terminal enzymes acting in the conversion of PGH₂ to the active prostanoids downstream of COX have been cloned and characterized. These enzymes include PGE synthase (PGES) for PGE₂, PGDS for PGD₂, PGFS for PGF₂α, PGIS for PGI₂, and TXS for TXA₂. These terminal PG synthases are known to be functionally linked with preferential COX isozymes [30]. In the final step of PGE₂ biosynthesis downstream of COX, PGES specifically catalyzes the conversion of PGH₂ to PGE₂. To date, at least three individual forms of PGES, cytosolic PGES (cPGES), microsomal PGES (mPGES)-1, and mPGES-2, have been cloned and characterized [31-33]. cPGES is largely believed to contribute physiologically to the production of PGE₂ for the maintenance of homeostasis based on the fact that it is constitutively expressed and functionally coupled with COX-1 in the cytosol under basal conditions in various cells and tissues [32]. mPGES-1, on the other hand, shows coordinated induction with COX-2 by inflammatory stimuli in various cells and tissues [31, 34]. Because of its inducible nature, it has been of interest to investigate the role of mPGES-1 in inflammatory diseases. mPGES-2 has a catalytic glutaredoxin/thioredoxin-like domain and is activated by various thiol reagents. Like cPGES-1, mPGES-2 is also constitutively expressed in various cells and tissues. However, the fact that it is functionally coupled with both COX-1 and COX-2 [35] indicates that mPGES-2 may play a role in the production of PGE₂ not only in homeostasis but also in pathological conditions.
Microsomal prostaglandin E synthase-1 in articular cells and tissues

mPGES-1, originally known as microsomal glutathione S-transferase 1-like 1 (MGST1-L1), is a glutathione-dependent enzyme that shows coordinated induction with COX-2 by inflammatory stimuli in various cells and tissues [31, 34]. mPGES-1 has been considered the most prominent PGES isozyme to be targeted in inflammatory diseases including OA. We and others have reported that articular chondrocytes from OA patients express mPGES-1 after stimulation with the proinflammatory cytokines IL-1β or TNF-α [36, 37]. In addition, the pattern of mPGES-1 expression by cytokine-activated synovial fibroblasts was shown to be similar to that observed in OA chondrocytes [38]. mPGES-1 protein (immunoreactivity) was also detected in both the chondrocytes and synovial lining cells in OA patients. These observations indicate that overexpression of mPGES-1 in articular tissues such as synovium and cartilage may be a crucial contributing factor to the development of chronic articular inflammation in OA patients.

The roles of mPGES-1 have also been demonstrated by several studies using mPGES-1-deficient mice in experimental models of arthritis. We and others have demonstrated that mPGES-1-deficient mice are resistant to CIA [39] and show a marked reduction in anti-type II collagen antibodies [40], and decreased pain response in models of inflammatory pain and neuropathic pain [39-41]. A study using several mouse models of skeletal disorders with mPGES-1-deficient mice revealed that mPGES-1 was indispensable for bone repair via proliferation of chondrocytes [42]. Conversely, studies have also shown that mPGES-1 was not essential for the skeleton under normal physiological conditions, nor did it play a role in the pathophysiological conditions of bone loss (due to ovariectomy or to unloading) or during stress-induced OA. Since studies using mPGES-1-deficient mice reveal resistance to some symptoms of inflammatory arthritis, selective mPGES-1 inhibitors could be promising targets for the treatment of OA and other forms of arthritis.

Prostaglandin E2 and its receptors

Prostanoids exert a variety of physiological and pathophysiological actions via their respective receptors expressed on target cells. The PG and TX receptors are G protein-coupled receptors with seven transmembrane domains. They are comprised of EP for PGE2, DP for PGD2, FP for PGF2α, IP for PGI2 and TP for TXA2 [43]. In addition, another PGD receptor, CRTH2 (also known as DP2), was identified as the chemoattractant receptor-
homologous molecule expressed on Th2 cells [44]. Expression of these receptors depends on the cell type they are expressed on, leading to alterations in the specificity and physiological functions of the final active products.

PGE_2_ exists in a wide variety of cells and tissues, and plays important roles in various physiological functions in addition to its role as a major mediator of inflammation. High concentrations of PGE_2_ have been detected in the synovial fluid of OA patients and it is well established that it is a key mediator of cartilage degradation. In growth plate chondrocytes, PGE_2_ has been shown to increase DNA synthesis and inhibit collagen synthesis [45]. It has also been shown to increase matrix metalloproteinase (MMP) production in human OA cartilage explants [19], articular chondrocytes [46] and synovial fibroblasts [47]. PGE_2_ also potentiates inflammation by promoting the expression of the proinflammatory cytokine IL-1\beta [48].

As mentioned above, the preferential production of PGE_2_ results from the sequential activation of two cytokine-inducible enzymes, COX-2 and mPGES-1. We previously reported that increased production of PGE_2_ in primary cultured chondrocytes from OA patients [36] and human rheumatoid arthritis synovial fibroblasts [49, 50] is due to induction of mPGES-1 by proinflammatory cytokines.

PGE_2_ exerts its effects via four EP subtypes, EP_1, EP_2, EP_3, and EP_4. There are differences in the downstream signalling pathways of the EP subtypes. EP_1 is coupled with PLC/PI3 signalling and stimulates the release of intracellular calcium, while EP_2 and EP_4 increase cAMP by activation of adenylate cyclase via coupling to G_s-type G protein [51]. Although EP_3 has variants that mediate multiple signalling pathways, it generally causes the inhibition of cAMP via G_i-type G protein [52].

EP_2 and EP_4 have been shown to be expressed at higher levels in knee cartilage of OA patients [53]. Specific agonists and antagonists targeting each EP have been developed and used for research [54]. A study using selective EP agonists for individual EP_{1-4} subtypes clearly demonstrated in OA synovial fibroblasts that PGE_2_ regulates the production of IL-1\beta-induced IL-6, macrophage colony stimulating factor, and vascular endothelial growth factor through the activation of EP_2 and EP_4 with an increase in intracellular cAMP [55]. A similar regulation of TNF-\alpha-induced IL-6 and MMP expression was also recently reported in synovial fibroblasts [56].

In OA chondrocytes, PGE_2_ has been shown to inhibit proteoglycan synthesis and stimulate matrix degradation via EP_4 [57]. A recent study also showed that COX-2-derived PGE_2_ signals via upregulation of EP_2 and downregulation of EP_3 to increase intracellular cAMP, and activate the
protein kinase A and phosphatidylinositol 3-kinase/Akt pathways, regulating shear stress-induced IL-6 expression in chondrocytes [58]. Hence, PGE$_2$-EP signalling may play a pivotal role in the pathophysiology of chronic inflammation related to OA.

Apart from EPs, recent studies suggest the possible impact of the PGI$_2$-IP system in experimental models of acute pain [59]. There is a need for detailed roles of other prostanoid receptors in OA to be further investigated.

**Prostaglandin E$_2$ inhibition in osteoarthritis**

PGE$_2$ inhibition has long been the prime therapeutic target to overcome the destructive and inflammatory mechanisms associated with OA. Inhibitors of COX-2 exert antiinflammatory effects and pain relief by inhibiting both PGE$_2$ and PGI$_2$. Traditional NSAIDs such as indomethacin and ibuprofen are clinically effective for the management of pain associated with OA and related diseases such as rheumatoid arthritis. However, traditional non-selective NSAIDs have been associated with a number of gastrointestinal and renal side effects due to their inhibitory effects on both COX-1 and COX-2, resulting in inhibition of PG and TX production [60]. Selective COX-2 inhibitors were therefore developed with the aim of reducing these side effects of non-selective NSAIDs [61]. Selective COX-2 inhibitors, or COXIBs, have improved gastrointestinal toxicity and similar efficacy to the non-selective NSAIDs in OA patients [62-64]. The VIGOR (Vioxx GI Outcomes Research) study showed that treatment with rofecoxib resulted in significantly less clinically important upper gastrointestinal events than treatment with naproxen [62]. However, long term use of these COXIBs has been shown to be associated with increased risk of myocardial infarction and thrombosis [65]. Therefore, rofecoxib was withdrawn worldwide in 2004, and in 2005 valdecoxib was withdrawn by the United States Food and Drug Administration (FDA) with concerns regarding increased cardiovascular side effects. Another COXIB, celecoxib, is presently under the FDA alert [66].

A possible primary mechanism related to the cardiovascular side effects associated with selective COX-2 inhibitors is their ability to inhibit not only PGE$_2$ production but also PGI$_2$ (derived from endothelial COX-2), which plays a key role in the regulation of thrombogenesis [67]. Hence, a more specific inhibition of PGE$_2$ production via inhibition of mPGES-1 would seem to be a more relevant approach. Indeed, within the AA metabolic pathway, COX-2 is located relatively higher than MPGES-1, resulting in the inhibition of all PGs. By inhibiting mPGES-1, hypothetically only PGE$_2$ can be blocked. Studies performed in COX-2 and mPGES-1 transgenic mice fully support this hypothesis. Indeed, mice with COX-2 genetic deletion, mutation,
or treatment with celecoxib develop thrombosis and hypertension. However, mPGES-1-deficient mice exhibit significant reduction in PGE$_2$ production and increased PGI$_2$ production with no alterations in blood pressure or thrombogenesis when fed a normal or high salt diet [68]. In addition, loss of mPGES-1 in mice retards atherogenesis associated with decreased PGE$_2$ production, increased PGI$_2$ production and no change in TX production [69]. Furthermore, mPGES-1-deficient mice in experimental models of arthritis are resistant to cartilage destruction associated with marked reduction in inflammation and pain response [39-41].

**Conclusion**

mPGES-1 seems to be an attractive therapeutic target for counteracting OA while avoiding the cardiovascular side effects associated with COX-2 inhibition. However, these assumptions can only be proven when a specific potent inhibitor of mPGES-1 is tested in a human clinical setting. It will be very interesting to see how effectively an mPGES-1 inhibitor is able to mimic the results observed in mPGES-1-deficient mice. In addition to mPGES-1, targeting EP receptor subtypes is another promising alternative approach.

**References**