

Bradykinin and its role in osteoarthritis

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SUMMARY

Osteoarthritis (OA), the most common joint disorder, is a disease involving all the articular structures. It presents both degenerative and inflammatory aspects. Recently, the important role of Bradykinin (BK), a phlogistic mediator, has been proposed in the pathophysiology of OA. In our review, we summarized the currently available information on the mechanisms of action of BK in OA by linking its B₂ receptors. Then, we analyzed the data about the effects of BK in synoviocytes and chondrocytes cultures. Furthermore, we described the action of B₂ receptor antagonists (Icatibant and Fasitibant), presenting them as new promising symptom-and-disease-modifying agents in the treatment of OA. However, more *in vitro*, animal model and clinical studies, are needed to better understand the mechanisms of action as well as the efficacy and tolerability of the B₂ receptor antagonists in OA.

Key words: Bradykinin B₂ receptor antagonists, Bradykinin, Fasitibant, Icatibant, osteoarthritis.

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■ INTRODUCTION

Osteoarthritis (OA) is the most prevalent disease of articular joints in older adults in industrialized countries (1). The number of people with symptomatic OA is likely to increase due to the aging of the population. This disease plays an important medical and social role, causing deformity and joint disability, especially when it affects the knee and hip (2).

The main features of OA are degeneration and loss of articular cartilage, but all the joint components, including the bone, synovial lining, ligaments, tendons and periarticular tissue undergo structural and functional alterations during the course of OA progression (3). Cartilage breakdown is attributable to an imbalance between synthetic (anabolic) and resorptive (catabolic) activities of the resident chondrocytes. The catabolic processes exceed anabolic processes leading to a net decrease in extracellular matrix components (ECM) (type II collagen and proteoglycans) (3-6). Although the pathogenesis of OA is not fully understood, current knowledge indicates that many catabolic and proinflammatory factors contribute to the degradative cascade in OA. The spectrum of these factors includes

different cytokines such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor α (TNF- α), chemokines such as interleukin-8 (IL-8), numerous metalloproteinases (MMP) (MMP-3 MMP-9 MMP-13), prostaglandin E₂ (PGE₂) and nitric oxide (NO) (5). Recently, several evidences suggest the involvement of bradykinin (BK) and B₂ receptors in the pathophysiology of OA (7, 8). The aim of this study is to summarize preclinical and clinical evidences, which supports the hypothesis for a role of BK in OA pathogenesis.

■ BRADYKININ, KININS AND THEIR RECEPTORS

Kinins are peptides involved in both acute and chronic inflammation inducing pain, edema formation, vasodilation, and prostaglandins synthesis.

They are produced by specific proteases such as plasma and tissue kallikrein. Kallikrein-kinin system is activated by coagulation factor XII, a self-activating protease that converts prekallikrein in kallikrein. Kallikrein, in turn, activates the coagulation factor XII causing a positive feedback. Two different cascades and specific pre-

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cursors produce kinins: one in the plasma, the high molecular weight kininogen, and another in the tissues, the low molecular weight kininogen (9) (Fig. 1).

The high molecular weight kininogen generates, by plasma kallikrein, BK, a nonapeptide whose aminoacid sequences is Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, whereas low molecular weight kininogen leads to the formation of kallidin (Lys-BK), having amino-terminal additional lysine residual.

BK and Lys-BK are rapidly degraded by different enzymes, such as the carboxypeptidase M and N, which cleave off the carboxy-terminal arginine to produce the active desArg metabolites (9, 10) (Fig. 1). There are at least two kinins receptors, B_1 and B_2 , which belong to the Class I of G protein coupled receptors (IUPHAR) (11). The B_1 receptors mediate the effect of C-terminal desArg metabolites, while the B_2 receptors mediate the effect of BK and Lys-BK (12) (Fig. 1).

A recent study conducted in 2012 by Chen et al. (13) analyzed the role of +9/-9 polymorphisms of B_2 receptor (BDKRB₂) gene in 245 patients with primary knee OA and 264 healthy controls. The Authors reported that -9/-9 genotype had a significantly in-

creased risk for knee OA, when compared with +9/+9 genotype, and that BDKRB₂ polymorphism was also associated with OA radiographic severity. This finding suggests that BDKRB₂ +9/-9 polymorphisms may be used as a genetic marker for the onset and development of OA.

■ BRADYKININ IN THE SYNOVIAL FLUID OF OSTEOARTHRITIS PATIENTS

Initially evidence of the presence of BK in the synovial fluid of patients affected by arthritis of different etiologies, including OA, was early presented in 1967 by Melmon et al. (14). Later, Eisen (15) found the presence of BK in 33 out of 82 synovial fluids of patients with rheumatoid arthritis (RA) and in 2 out of 27 fluids from joint effusions caused by OA or trauma.

In 1992, Bond et al. (16) reported that the synovial BK content of patients with RA ranged between 1 and 60 nM. Later, Nishimura et al. (17) demonstrated the high rate of BK in patients with internal derangements and OA of temporomandibular joint and, for this reason, BK might be a useful index of the degree of synovitis. A recent preliminary study conducted by Meini et al. (18) quantified BK levels in knee synovial fluids of patients with gonarthrosis, finding the basal BK content ranging between 281 and 563 pg/mL (mean 422) and generated BK content ranging 2591-4264 pg/mL (mean 3427). Moreover, a significant positive correlation was found between BK levels, both the basal and generated ones, and the contents of IL-6, one of the most important cytokines involving in OA.

■ BRADIKININ AND B_2 RECEPTORS IN HYPERALGESIA AND INFLAMMATION

The algogenic and proinflammatory activity of BK is linked to the activation and sensitization of nociceptive afferent nerve

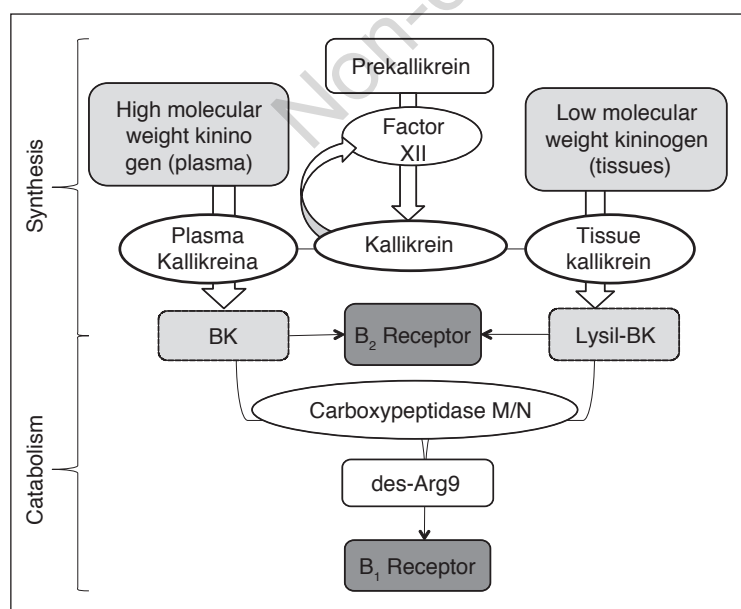


Figure 1 - Synthesis and catabolism of Bradykinin.

terminals, as demonstrated by Steranka et al. in 1988 (19). BK, through B₂ receptor interaction, activates the phospholipase C (PLC) with consequent inositol phosphate (IP) hydrolysis and diacylglycerol (DAG) production, which in turn potentiates the activation and increases the expression of vanilloid TRPV1 receptors (19-25).

BK has also been shown to evoke calcitonin gene related peptide (CGRP) release in knee joints of mice, which spontaneously developed OA, and to produce a significantly higher release of PGE₂ in joints of animals treated with collagenase as a model of OA (26). In 1993, Gotoh et al. (27) showed that the intra-articular administration of BK could induce hyperalgesia in rats. Consecutive studies of Davis and Perkins (28, 29) demonstrated that the hyperalgesia, produced by intra-articular administration of substances such as capsaicin or substance P that stimulate the nociceptive sensory fibers, could be blocked by systemic treatment with Icatibant, a B₂ receptor antagonist (30). The intra-articular injection of the synthetic peptide of BK into dog knee (500 µg in 1 mL) caused local warmth and swelling within a few minutes of administration, as a consequence of hyperemia of synovial membranes and increased synovial fluid demonstrating the involvement of BK in inflammatory events (14). Later studies in the rat synovium demonstrated that intra-articular administration of BK induced plasma extravasation and neutrophils accumulation (31-33) more potently than other inflammatory mediators such as substance P, histamine, and CGRP (34). BK-induced plasma extravasation resulted due to a direct stimulation of B₂-type BK receptor, which is likely to be located on the vascular endothelial cells and/or smooth muscle and could be inhibited by the selective B₂ receptor antagonist (35).

■ KININS EFFECTS ON SYNOVIOCYTES

The synovial membrane presents in its superficial layer a unique cellular lining, the synovial intima, which is one of three cells

deep. The synovial intima contains two types of synoviocytes. Type A synoviocytes, which are derived from blood-borne mononuclear cells, can be considered as resident macrophages owing to their ability to actively phagocytose cell debris and wastes in the joint cavity and antigen-presenting ability. Type B synoviocytes cells are involved in the production of specialized matrix constituents, including hyaluronan, collagens, and fibronectin for the intimal interstitium and synovial fluid (36). Bathon et al. (37), in 1991, first characterized the number affinity and receptor subtype of specific kinin binding sites in intact human synovial tissue and in cultured human synovial cells. Furthermore, they examined the effect of IL-1 treatment on these binding parameters. Specific saturable tritiated BK binding sites in intact synovia were identified by autoradiographic localization and were present in much higher density in RA than in OA synovia. The kinin binding site on the synovial cells belonged to the B₂ class of kinin receptors. The treatment with IL-1 resulted to enhance specific tritiated BK binding in synovial cells. The presence of B₂ receptors on human synoviocytes was further confirmed by Uhl et al. (38) and Cassim et al. (39), who performed a study to localize B₂ receptors. These receptors were observed in the synovial lining cells, fibroblasts, and endothelial lining cells of blood vessels, whereas no evidence supported the presence of the B₁ receptor subtype.

Bellucci et al. (40) conducted a study to investigate new proinflammatory actions by BK in human fibroblast-like synoviocytes. In particular, the Authors focused on the ability of BK to induce IL-6 and IL-8 production by synoviocytes and the mechanism involved. They showed that BK, after a long-term incubation, was able to increase the release of IL-6 and IL-8 from human synoviocytes in a concentration-dependent manner. Furthermore, the Authors presented pharmacological evidence that PLC and nuclear factor kappaB (NF-κB) activations were involved in the BK-mediated release of IL-8, as previously described for IL-6 in other studies.

Moreover, BK induced the release of arachidonic acid (AA), and PGE₁ and F were synergistically potentiated when the cells were cultured in the presence of higher concentrations of serum and when the BK was co-administered with PGE₁, thus suggesting that some other factors may be involved in activating the adenylylcyclase pathways in human synovial fibroblasts (41, 42).

Meini et al. (43) demonstrated that the incubation of synoviocytes with BK induced a sustained production of PGE₂ and transient cyclooxygenase-2 (COX-2) gene expression. The co-incubation of cells with BK and IL-1 β induced a greater increase in released PGE₂ and COX-2 gene and protein expression, indicating a synergistic rather than an additive effect. These potentiating effects of BK on PGE₂ production and increased COX-2 expression produced by IL-1 β were B₂-receptor-mediated because Fasitibant could be prevent them.

■ KININS EFFECTS ON CHONDROCYTES

In 2011, Meini et al. (44) conducted a study on rat and human articular chondrocytes to define the pharmacological profile of BK, its receptors, and some of its analogs and antagonists, especially the recent non-peptide B₂ receptor antagonist Fasitibant or MEN16132. They used tritiated BK to pharmacologically characterize the BK B₂ receptor. They compared the power of MEN16132 with Icatibant, by measuring the accumulation of IP and the release of IL-6 and IL-8 induced by BK. The density of BK receptors was high and greater in human chondrocytes, when compared with rat chondrocytes. The inhibitors MEN16132 and Icatibant showed similar binding affinity. The Authors also observed greater accumulation of IP induced by BK in human chondrocytes, when compared with those in rats and the antagonist MEN16132 was more potent than Icatibant in inhibiting accumulation of IP. Furthermore, after incubation for 24 h in cultured human chondrocytes, BK increased the release of IL-6

and IL-8, an effect which was neutralized by MEN16132, but not by the antagonist of the receptor B₁. Furthermore, the Authors investigated the mechanisms involved in the IL-6 and IL-8 release induced by BK in human chondrocytes by pre-treating these cells with different inhibitors. Human chondrocytes were exposed to pretreatment with the nonselective COX inhibitor indomethacin, the nonselective lipoxygenase nordihydroguaiaretic acid (NDGA), or the glucocorticoid dexamethasone before BK stimulation. The release of IL-6 induced by BK was partially inhibited by indomethacin and NDGA, but it was not the same for the release of IL-8.

Dexamethasone has been shown to reduce both the release of IL-6 and IL-8 stimulated by BK.

In conclusion, this study demonstrated the important role of BK in inflammatory and degenerative process of OA and advanced the hypothesis of a possible use of BK B₂ receptor antagonists in the treatment of OA.

■ BRADIKININ B₂ RECEPTOR ANTAGONISTS AND THEIR CLINICAL IMPLICATIONS

Considering the important role of BK in inflammation and its activity on different kinds of joint cells, B₂ receptor antagonists may represent a new therapeutic strategy in OA (45). Icatibant is a synthetic decapeptide and a potent, stable, specific, and long-acting antagonist of the BK B₂ receptor; it is currently used for angioedema attacks (46, 47) (Fig. 2).

Over the years, much of the pharmaceutical research was aimed at the identification of nonpeptidic small molecules, which are more useful from a clinical point of view (48-50). These studies led to the synthesis of a nonpeptide B₂ receptor antagonist, known as MEN16132 or Fasitibant (Fig. 2). Different studies were conducted to investigate the pharmacological characterization (affinity, selectivity, and antagonist potency) of MEN16132 in various animal and human tissues (i.e. mouse lung and

ileum smooth muscle, human and rat urinary bladder, rabbit and pig ileal smooth muscle) (51-55).

MEN16132 is a new potent and selective nonpeptide BK B₂ receptor antagonist (51-55).

Meini et al. (46) recently compared the different pharmacological effects of Icatibant and Fasitibant on human synoviocytes BK B₂ receptor.

First, the reversibility of the antagonist action in blocking B₂ receptors was compared through functional experiments (IP accumulation assay), and then through radioligand binding experiments, the affinities of the two antagonists were compared using a panel of point-mutated receptors.

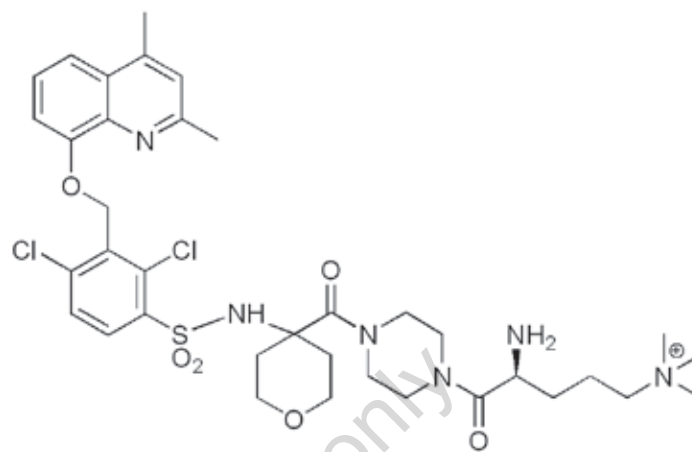
The results of the study showed that MEN16132 was more potent than Icatibant with regard to its slower reversibility from the B₂ receptor compartment, and this was due to the interaction of MEN16132 at a deeper level in the transmembrane regions of the receptor.

In addition to the *in vitro* studies, clinical studies were also conducted to assess the efficacy and tolerability of BK B₂ receptor antagonists.

In 2004, Flechtenmacher et al. (56) performed a double-blind placebo-controlled Phase II study on 58 patients with symptomatic knee OA in which a single intra-articular injection of the antagonist Icatibant (90 µgr/mL) reduced pain intensity more effectively than the placebo. Furthermore, the Authors reported that the analgesic activity of Icatibant was more significant on the pain perceived during activity than on pain at rest. This evidence was later confirmed by another randomized double-blind placebo-controlled study in 2009 (57).

With regard to the efficacy of MEN16132, preclinical data are being confirmed by an ongoing clinical study (multicenter randomized, double-blind, placebo-controlled), named ALBATROSS, on patients with knee OA treated with intra-articular administration of MEN16132. This study is registered in Clinical Trials database (www.ClinicalTrials.gov; number: NCT01091116).

Fasitibant



Icatibant

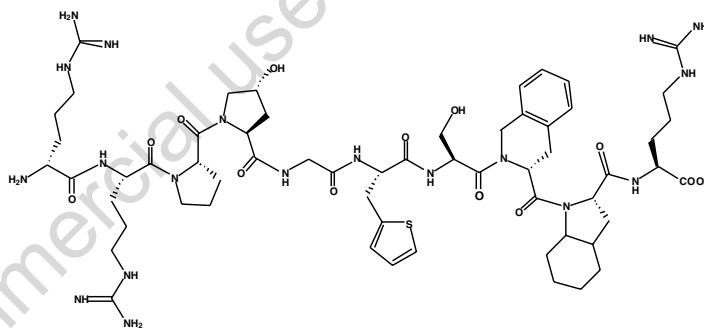


Figure 2 - Chemical structure of the antagonists of B₂ receptor (Icatibant and Fasitibant).

■ CONCLUSIONS

In conclusion, our literature review demonstrated that BK is an important mediator of articular pain and inflammation, which stimulates the release of inflammatory and catabolic cytokines by articular chondrocytes.

These findings, in agreement with preclinical studies, lead to the notion that the BK B₂ receptor antagonists could be important symptomatic drugs in the treatment of OA, capable of controlling pain and improving joint function.

Furthermore, BK B₂ receptor antagonists, acting directly on chondrocyte and reducing the release of proinflammatory cytokines (IL-6, IL-8), may also represent new disease modifying osteoarthritis drugs

(DMOADs) for the treatment of OA, decreasing or stopping the progression of the disease (52, 53).

BK B₂ receptor antagonists therefore appear as a promising therapeutic strategy in OA.

However, further *in vitro*, animal model, and clinical studies, are needed to better understand the mechanisms of action as well as the efficacy and tolerability of the B₂ receptor antagonists in OA (58, 59).

Conflict of interests: the authors report no conflict of interests.

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