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Review

PDE4 inhibition in rheumatoid arthritis

Rasolonirina Lea Corinne[1], Mopa Alina Sooro[2], Wang Jingqi[1], Ji Yingying[1], Xu Yungen[3,*,$] and Gong Guoqing[1,*,#]

[1]Department of Pharmacology; [2]Jiangsu Key Laboratory of New Drug Screening;
[3]Department of Medicinal Chemistry, China Pharmaceutical University,
No.639 Longmian Road, Jiangning District, Nanjing, Jiangsu, China.

ABSTRACT

Rheumatoid arthritis (RA) appears to be a complicated disease as its etiology still remains elusive despite the numerous advances and knowledge concerning its progression. Soluble tumor necrosis factor (TNF) receptors are mainly found in high concentration in synovial fluid and serum of patients with rheumatoid arthritis. Thus excess of TNF-α relative to this high level of soluble TNF receptors amplify joint inflammation. Therapies currently employed for RA treatment include the administration of non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatics drugs (DMARDs) including other newer classes of drugs which antagonize specific markers involved in the pathogenesis of RA. Since Infliximab has been approved by the United States Food and Drug Administration (FDA) in 1992, anti-cytokines therapy has continually been proven efficient and effective despite its high cost. The use of phosphodiesterase (PDE4) inhibitors is an emerging therapy in the class of anti-cytokines drug therapies and it is increasingly being demonstrated to be the promising treatment strategy for rheumatoid joint with a safer side effect profile compared to some of the currently approved medications. The discovery of C-terminal regulatory element CR3 (Control Region 3) in PDE4 structure has further brought advanced insight into the treatment of inflammatory joint disease. PDE4 inhibition affects the regulatory role of p38 MAPKs in cytokine production and suppresses the overexpression of cytokines through cAMP, PKA and NF-κB route. Indeed, current research demonstrates PDE4 as an important, although under-exploited, molecular target for anti-rheumatoid arthritis. Therefore, in this review we provide a comprehensive overview of PDE4 activity and show how its inhibition is crucial in the treatment of rheumatoid arthritis (RA) and how this class of drugs can be further developed for enhanced effectiveness.

KEYWORDS: PDE4 inhibitors, rheumatoid arthritis, cAMP, lipopolysaccharide, TNF-α.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, destructive inflammatory arthropathy manifested by articular and extra-articular features, resulting in joint damages mediated by cytokines and chemokines among others [1, 2]. RA affects approximately 1-2% of the population worldwide [3]. Incidence increases with age and the risk of incidence appears to be greatest for women between 40 and 50 years and for men it occurs even at a later age. The disease is therefore termed as an age-related immune disease [4]. Females have been reported to be three times more susceptible to RA than men [5]. Recent research reports that American Indians and Alaska natives show high vulnerability to RA owing to genetic reasons and recent studies reported that the Japanese and Chinese show less prevalence [6].

*Corresponding author
[#]gonggq@hotmail.com
[$]xyg64@126.com

PDE4 enzymes have attracted great interest because of the possibility of deploying selective inhibitors of these enzymes as therapeutics agents in many disease conditions including RA. PDE4 enzymes are a family of 3, 5-cyclic adenosine monophosphate (cAMP)-specific phosphodiesterase, encoded by four genes (PDE A-D) that have promoters and give rise to a multitude of proteins by alternative splicing [7]. Despite the simultaneous expression of the different subtypes of PDE4, it has been recently reported that one of the three PDE4 genes expressed in mouse peritoneal macrophages is involved in the control of Toll-like receptor (TLR). Lipopolysaccharide/Toll-like receptor 4 (LPS/TLR4) activation causes a major up-regulation of PDE4B and more than 25 human PDE4 isoforms (from 50 to 125 kDa) have been identified [8]. The PDE4 activity occurs due to stimulation of G protein-coupled receptors (GPCRs) which are the integral cell membrane proteins that activate adenyl cyclase (AC) which in turn converts adenosine triphosphate (ATP) into cAMP. Increased intracellular cAMP levels have downstream effects that include activation of cyclic nucleotide-gated ion channels, exchange proteins activated by cAMP (Epacs), and protein kinase A (PKA). Cyclic AMP then converts protein kinase A from inactive to active form and this mechanism is known as the cAMP-dependent pathway that activates key proteins within the nucleus to initiate or alter a cellular response [9, 10]. PDE4 is highly expressed by immunologic cells including dendritic cells, T cells, macrophages, and monocytes. Inhibitors of phosphodiesterase-4 are anti-inflammatory and immunosuppressive because they prevent the breakdown of cAMP which controls gene expression through PKA-mediated phosphorylation of the constitutively DNA-bound transcription factor, the cyclic AMP response element binding protein (CREB). CREB signaling occurs through a multistep pathway in which binding of hormones or neurotransmitters to G protein-coupled cell surface receptors activates AC and catalyzes the production of cAMP. Elevation of cAMP levels leads to activation and translocation of the catalytic subunits of PKA to the nucleus, where they catalyze the phosphorylation of CREB at Ser-133, which activates CREB [11]. The effects of cAMP are mediated through interactions of cAMP with PKA, Epac or ion channels. Epac1 (cAMP-GEFI) and Epac2 (cAMP-GEFII) are closely related guanine nucleotide exchange factors (GEFs) for the small GTPase Rap1, which are directly regulated by cAMP. Rap1 and Rap2 have been shown to control a number of cellular processes [10, 12]. cAMP-regulated chemotaxis and integrin-dependent adhesions of vascular endothelial cells to extracellular matrix proteins are coordinated by both PKA and Epac. As it is not clear whether cAMP is directly regulated through PKA or through Epacs in monocytes and macrophages, studies have shown that although Epac1 is expressed in human peripheral monocytes and activates Rap1, cAMP modulates most monocyte immune functions by activation of PKA and not through the Epac1-Rap1 [12, 13]. PDE4 inhibition elevates intracellular cAMP levels, which inhibit the NF-κB pathway, resulting in the down-regulation of the inflammatory responses by reducing the expression of TNF-α and other proinflammatory cytokines in RA [14].

Rheumatoid arthritis synovial fibroblasts (RASFs) elaborate and secrete a wide range of proinflammatory mediators, including cytokines (interleukin-1 IL-6, IL-10, IL-12, IL-17 tumor necrosis factor alpha or TNF-α), chemokines (IL-8, IL-10, MCP-1, RANTES), and growth factors (GM-CSF, G-CSF, and TGF-β). They stimulate changes such angiogenesis, inflammation, chemoattraction and proliferation of synoviocytes and bone erosion [15]. Furthermore, various effectors including protein kinases such MAPKs (mitogen-activated protein kinases) are involved in the regulation of inflammatory responses as well as in the modulation of the mRNA stability of many cytokines at the transcriptional level. MAPK pathway has been associated with RA through its mediation of TNF production, which is one of the inflammatory mediators in arthritis [16, 17]. Release of cytokines is also influenced by monocyte LPS-stimulation. LPS is one of the most potent inducers of cytokines production. There is considerable evidence from rodent models that activation of the Toll-like 2 and/or Toll-like 4 receptors (TLR4 and/or TLR2) can induce or exacerbate inflammatory arthritis and subsequently act in the up-regulation of inflammatory response in RA and that the RA synovial membrane cells potentially release factors that can stimulate TLR signaling [18]. Recently, progress has been achieved in the development of phosphodiesterase inhibitors for a variety of conditions. Thus in this review, we

first present information regarding the structure of PDE4 and then elucidate the PDE4 inhibition in rheumatoid synovial process.

2. Structure and functional analysis of PDE4

2.1. Splice variants

PDE4B subtype is believed to play a central role in inflammation, being the predominant subtype in monocytes and neutrophils. In addition, it appears to be the major PDE4 in macrophages and depletion of PDE4 in mice damage the production of TNFα in response to inflammatory stimuli [8]. PDE4B comprises three domains: a N-terminal regulatory domain, a catalytic domain of about 300 amino acids, and a C-terminal domain. The catalytic domain is the most conserved domain among the PDE families and consists of 17 α-helices [19, 20]. It has been investigated as an attractive and excellent therapeutic target due to its sensitivity to selective inhibitors. A high throughput screening study resulted in the identification of the lead compound which exhibited significant PDE4B inhibition. In the study, the authors further investigated the structure-activity relationship to optimize this compound which afforded a series of potent PDE4B inhibitors with more than 100-fold selectivity over the PDE4D isoenzyme [8, 21]. The catalytic domain (approximately 300 amino acids) is highly conserved in all PDE isoenzymes [22]. These proteins contain unique amino acid regions called upstream conserved region 1 (UCR1) with 55 amino acids and UCR2 with 97 amino acids [22, 23]. These PDE4 isoforms are categorized as long, short or super short depending on the presence and number of upstream conserved regions [24]. The long forms contain UCR1, linker region 1 (LR1), UCR2, LR2 and the catalytic domain. The short form and the super-short variant have LR1-UCR2-LR2 and UCR2 (truncated)-LR2 in the N-terminal region; the short forms which lack UCR1, and the super-short forms which contain only the C-terminal portion of UCR1 and UCR2 are functional modules. The linker regions, LR1 of approximately 33 residues and LR2 of approximately 28 residues, encode PDE4 subfamily-specific sequences that serve to connect UCR1 to UCR2 and UCR2 to the catalytic unit [25, 26]. Exons 4 and 5 contribute to encoding LR1 while exons 8 and 9 contribute to encoding LR2 [27]. UCR domains are known to provide a regulatory module which is able to influence the functionality of the catalytic unit. Thus the linker regions LR1 and LR2 may serve to confer subfamily-specific attributes on the regulation of catalytic activity [28]. UCR2 bears an auto inhibitory nature, a property inferred from observations that removal of a portion of this domain causes an increase in the catalytic activity of the enzyme; UCR1 contains PKA phosphorylation site. In short-term activation, long isoforms of PDE4 are regulated by phosphorylation, association to a protein or endogenous mediators; PKA-mediated phosphorylation of UCR1 allows rapid changes in PDE4 activity. The increase in PDE4 activity hydrolyses cAMP resulting in a return of cAMP levels to the basal state [27]. However, PDE4B isoform still remains an underexplored target. The amino acid of the PDE4 active site is identical in all PDE4 subtypes; therefore it is difficult to develop selective PDE4 inhibitors.

2.2. The novel PDE4 structure element CR

It has been found that PDE4 inhibitor showed the highest activity as well as selectivity. The comparison of the binding mode of the PDE4 inhibitor with the isoforms PDE4B and PDE4D complexes revealed that PDE4 inhibitors form additional hydrogen bonds with multiple water molecules as well as hydrophobic interactions with an active site called control region 3 (CR3) residue in PDE4B (Fig. 1), which were primarily responsible for PDE4B selectivity [28]. PDE4B selectivity can be achieved by capturing the C-terminal regulatory helix such that the active site is in a conformation that prevents cAMP binding [29]. PDE4B selectivity is driven by a single amino acid polymorphism in CR3 (Leu674 in PDE4B1 versus Gln594 in PDE4D) [22]. Other investigations reveal that PDE4B inhibitor compounds showed good binding affinity for PDE4B and PDE4D. The interactions of 6-gingerol with GLN443 in the active site and LEU502 in the CR3 of PDE4B were identified as the main interactions responsible for determining selectivity [24]. Within CR3 is located the multifunctional docking site lying across the opening of the catalytic pocket. As variation was observed in the mode of interaction with the core catalytic domain in these structures, it has been suggested that capture of CR3 by the catalytic pocket might reflect an autoinhibitory capping role that is

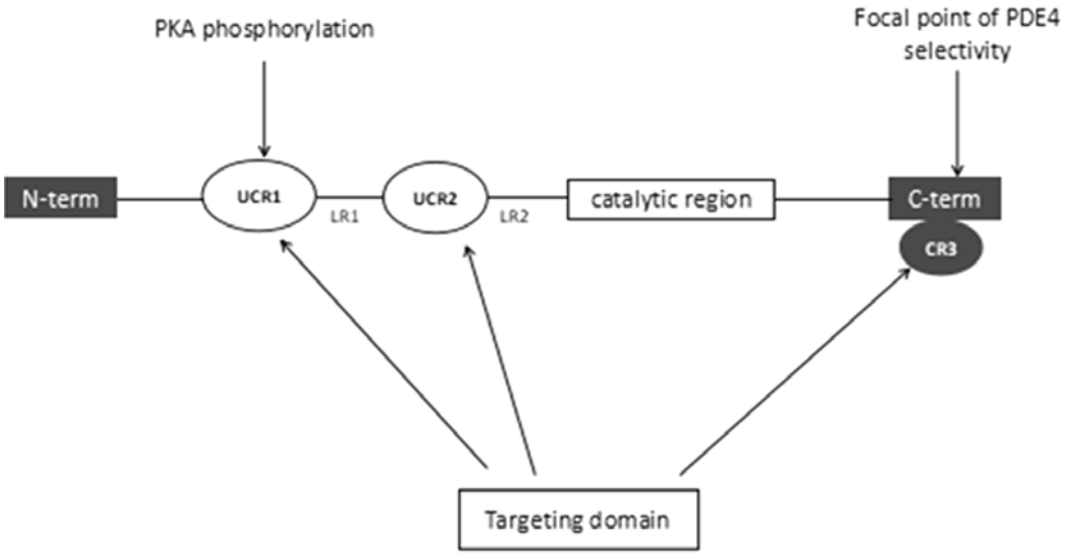

Fig. 1. Long form variant of PDE4 containing the C-terminal element CR3. **UCR:** Upstream conserved region; **LR** = Linker region; **CR3:** control region 3.

relevant to PDE4 function [23]. CR3 also forms weak interaction while catalytic domain and its length are variable in different PDE4. Indeed, generating selective PDE4B inhibitors is now possible through CR3 and by exploiting sequence differences outside of the catalytic domain. This corresponds to high degree of flexibility of CR3 whereby small molecule could be developed and may stabilize CR3 conformation [29, 20].

3. Implications of PDE4 inhibitors in rheumatoid arthritis

3.1. MAPK inhibition by PDE4 inhibitors

MAPKs are a group of serine/threonine residues belonging to target proteins and are among the most ancient signal transduction pathways which are conserved from yeast to mammals. They constitute the key regulators of a wide range of cellular processes, including gene expression, proliferation, and differentiation and programmed cell death [30]. The MAPK superfamily can be broadly divided into conventional and atypical MAPKs. The conventional MAPKs consist of three major groups: the p38 MAP kinases, the extracellular signal-regulate protein kinases (ERKs), and the c-Jun NH2 terminal kinases (JNKs). p38 MAPK and JNK signaling pathways coordinate extracellular stimuli with cellular responses, and they have been implicated in a range of cellular processes including the synthesis of proinflammatory cytokines TNF and IL-6 in *in vivo* studies and their downstream signaling leads to joint inflammation and bone degeneration [31, 32]. However, p38 MAPK is thought to be the main isoform involved in mediating cytokine production and among 4 different isoforms of p38 MAPK (α, β, γ, and δ), α and γ isoforms dominate in macrophage; β and γ isoforms are abundant in fibroblast and δ isoform in granulocyte [33]. Regulation of the components of the p38 MAPK signaling cascade has been considered crucial in inflammatory joint disease as it is essential in the mediation of synovial inflammation, cartilage damage, in inflammatory bone loss, and endothelial function. p38 MAPK contributes to the overexpression of proinflammatory cytokines, chemokines, MMPs and signaling enzymes such COX2 in inflamed tissue. This protein is further involved in mediating microvessel proliferation through its interaction with vascular endothelial growth factor (VEGF). p38 MAPK regulates adhesion molecule expression, such as E-selectin and VCAM-1 on endothelial cells, which regulates rolling and adhesion of leucocytes on the endothelium before transmigrating to the inflamed tissue. In synovial inflammation, p38 MAPK is involved in the synthesis of proinflammatory cytokines such as IL-1 and IL-6 and plays important roles in promoting RA by

inducing the production of IL-8, MMPs and adhesion molecules in the synovium. The produced cytokines in the synovium further induce MAPK activation in synovial fibroblasts and chondrocytes, leading to induction of MMP-1 and MMP-13 in chondrocytes [34-36]. Therefore, the therapeutic potential of downregulating p38 MAPK pathway results in the inhibition of cytokines and MMP production in RASF. Leflunomide was found to act on RASFs by downregulating MAPK signaling pathways through inhibition of COX-2 activity regulated by downregulation of p38 MAPK [37]. Recently, work on p38 MAPK inhibition tends to move towards enhancement of MAPK Phosphatase-1 (MKP-1) expression. MKP-1, a major regulatory molecule of p38 MAPK is transcriptionally up-regulated in corticosteroids by regulation of IL-8 through p38 MAPK/NF-κB signaling pathway or through p38 MAPK/mRNA stability cascades. It has been demonstrated that immunosuppressive gold aurothiomalate enhances MKP-1 expression resulting in the reduction of p38 MAPK activity [38, 39]. Recently, it has been discovered that PDE4 inhibitors block LPS-induced TNF-α production by downregulating p38 signaling pathway; however, this inhibition occurs in an indirect way as promotion of MKP-1 expression through PDE-4 inhibition leads to a decrease in p38 MAPK [40]. PDE4 inhibitors also have been demonstrated to inhibit the expression of microsomal prostaglandin E synthase-1 (mPGES-1), an enzyme synthesizing prostaglandin E_2 (PGE_2). Its expression may contribute to the pathogenesis of collagen-induced arthritis and mediate acute pain during inflammatory responses. PDE4 inhibition depends on the MKP-1 downregulation of JNK but not p38 MAPK [41].

3.2. Inhibition of cytokines and chemokines by PDE4 inhibitors

It is evident that overproduction of cytokines promotes and propagates the inflammation and rheumatoid joint. TNF-α is a predominant proinflammatory cytokine that induces several other cytokines in the proinflammatory cascade, including IL-1, IL-6, IL-8. TNF-α is also able to induce the release of chemokines that attract leukocytes and GM-CSF which is found in high levels in joints with rheumatoid arthritis and upregulates several integrins and adhesion molecules (intercellular adhesion molecule or ICAM and vascular cell adhesion molecule or VCAM) [42, 43]. The activation of TLR4/MyD88 signal transduction pathway subsequent to endotoxin LPS-TLR4 interaction is indispensable for the production of inflammatory mediators in synovial fluid resulting in NF-κB transcription factor activation and increased expression of inflammatory cytokines, chemokines, adhesion molecules and MMPs [44, 45]. TNF-α and IL-6 are also implicated in the regulation of RANKL (Receptor activator of nuclear factor kappa-B ligand) expression. These proinflammatory cytokines induce the activation of RANKL/RANK (OPG) pathway which is expressed in fibroblast-like synovium (FLS) or in activated synovial T-cell. RANKL/RANK also play a crucial role in osteoclast formation, maturation and induction of resorptive activity in RA [46]. IL-1α and IL-1β *in vitro* activate monocytes/ macrophages, induce fibroblast proliferation, stimulate chondrocytes and osteoclast, resulting in synovial pannus formation, cartilage breakdown and bone resorption, respectively, and may also transform the synovial fibroblast to turn into an aggressive phenotype. Cytokines upregulate cathepsin K, expressed in the synovial fibroblast at the cartilage-pannus junction and appear to be the only proteinase in its range capable of hydrolyzing type I and II collagen. Collagenase can also induce cartilage damage and induce the invasion of cartilage by synovial fibroblasts, although IL-1 and syndycan-4 make these synovial fibroblasts become aggressive and invasive cells, thus constituting a novel target for the treatment of arthritic cartilage damage [47-49]. The role of IL-12 in the pathogenesis of murine collagen-induced arthritis (CIA) results in severe arthritis that is associated with enhanced IFN-γ production [50]. Furthermore, IL-17 can drive T-cell-dependent erosive arthritis in the TNF-deficient and IL-1Ra knockout mice, and treatment of mice with CIA or adjuvant-induced arthritis (AIA) with neutralizing IL-17 antibody effectively inhibits cartilage destruction in these models of RA. IL-17 associated with IL-32 and TNFα facilitate increased production of other proinflammatory cytokines and chemokines; this association also affects the expression of IL-32 in FLS of RA patients leading to chronic inflammation and osteoplastic bone erosion [51].

Migration and invasion of activated FLS into cartilage and bone are critical events during invasive pannus

formation in RA synovium. Chemokines have been proven to play an important role in these events by ensuring leucocyte and neutrophil chemotaxis through the endothelial barrier into the inflamed synovium. IL-8 (CXCL8), epithelial–neutrophil activating protein-78 (ENA-78 or CXCL5), monocyte-chemoattractant protein-1 (MCP-1 or CCL2), RANTES (regulated upon activation, normal T-cell expressed and secreted or CCL5) and macrophage inflammatory protein-1α (MIP-1α or CCL3) are expressed in RA synovial tissue and in RA synovial fluids. IL-8 and ENA-78, which act on granulocytes, and MCP-1, RANTES and MIP-1α, which act primarily on monocytes and lymphocytes, are involved in the selective recruitment and activation of these cells [52-54]. TLR2, TLR3 and TLR4 have thus far been identified as being expressed on RASFs [55], in perivascular region of the joint, at the site of the attachment and invasion into cartilage/bone, and on synovial macrophages. TLR2 and its ligand PG have been detected in RA synovial fluid and PG activation induces intercellular adhesion molecule (ICAM-1), VEGF, IL-8, VCAM-1 and MMPs, which facilitate the interaction of RASFs with T lymphocytes through ligation of ICAM-1 to its binding partner leukocyte function associated antigen-1. Mediated by β1-integrin, TLR2 induces synovial RASFC migration and invasive process and promotes RA joint destruction by blocking the expression of MMP-3(stromelysin-1)/TIMP-3 and MMP-1(collagenase-1)/TIMP-3 (Tissue inhibitor of metalloproteinases) responsible for the erosive outgrowth in RA. CXCL10, a C-X-C chemokine is highly expressed and predominantly infiltrate the inflamed synovium. It is also expressed on effector T lymphocytes (Th1 cells) that mediate a type 1 inflammation such as RA [55-58]. Recently, it has been revealed that, in addition to its known chemotactic properties, CXCL10/ CXCR3 regulates FLS invasion and MMP-1 expression important in RASF invasiveness leading to the degeneration of articular cartilage and bone through cleavage of collagens I, II, VII and X [59, 60].

PDE4 inhibitors can have antiproliferative effect on human mononuclear cells in the presence of PGE2 which also has immunosuppressive properties due to the elevation of cAMP, leading to the TNF-α and IFN-γ suppression and an increase in IL-17. Indeed, this is probably important for preventing the recruitment of fibroblast in joint rheumatoid process [61]. FLS displays crucial role in the maintenance of joint mechanical stability and serves as a platform for regulation of multiple process in RA joint such as propagation of inflammation or maintenance of pannus. Proliferation of synovial fibroblasts in joints leads to pannus formation which contributes to cartilage destruction by the release of collagenolytic enzyme cells and other inflammatory cells arising from the junction between synovial tissue and cartilage in rheumatoid arthritis. Furthermore, PDE4 inhibitors have been recently demonstrated to exert antipannus forming effect by suppressing the production of cytokines such TNF-α, IL-1 and IL-12 and then blocking fibroblast proliferation [62, 63]. PDE4 inhibitors dose-dependently inhibit LPS-stimulated TNF-α release by enhancing cAMP, PKA and NFκB-dependant mechanisms and also inhibit T-cell proliferation and IFN-γ production. However, this suppression does not affect the inhibition of other cytokines such IL-6 or IL-10 [64, 65].

Actually, there is no PDE4 inhibitors conventionally approved for the treatment of rheumatoid arthritis. Otherwise, they remain to be promising after investigations revealing fewer side effects in the study of Apremilast (Oztela®) which has been approved by the FDA for the treatment of psoriasis and psoriatic arthritis [65, 66]. Besides, the actual studies of the effect of Apremilast do not highlight its efficacy on active RA in phase II clinic trials although it displays a good safety profile unlike the first generation PDE4 inhibitor Rolipram. Phase III clinical trials of Apremilast are currently under way and will provide more insights into its dosing and side effect profile. The most frequently reported adverse events have been headache, nausea and pharyngitis. Rolipram was also studied as an antidepressant several years before the discovery of its potent PDE4 inhibitory activity. Despite its potent anti-inflammatory effects *in vitro*, clinical trials were associated with unacceptably high rates of adverse events, specifically nausea and vomiting [7, 66, 67]. As researchers focus on new PDE4 inhibitors with fewer adverse effects, experimental compounds such CBS-3595 has been found to display fewer side effects in gastrointestinal tract and does not present hepatotoxicity [68].

4. Conclusion

In summary, properties of CR3 in PDE4 structure and the high selectivity in terms of PDE4B isoform would further promote the development of PDE4 inhibition in rheumatoid arthritis. The presence of cytokines and chemokines *in-situ* or during processes such as leucocyte chemotaxis and proliferation of pathogenic cells is relevant to the anti-cytokines activity of PDE4 inhibitors. Therefore, PDE4 inhibition is potentially the most effective strategy against rheumatoid arthritis at different stages. However, PDE4 inhibitor mechanisms in the enhancement of MKP-1 to p38 MAPK blockade still need to be elucidated.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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