p38 Mitogen-Activated Protein Kinase (MAPK) in Rheumatoid Arthritis

J. Westra* and P.C. Limburg

Department of Rheumatology and Pathology and Laboratory Medicine, University Medical Center Groningen, University of Groningen, The Netherlands

Abstract: The importance of p38 MAPK inhibitors as new drug for rheumatoid arthritis is reflected by the large number of compounds that has been developed over the last years. In this review new insights such as non-stressful activation of p38 MAPK, and the role of p38 MAPK in regulation of NF-κB recruitment are also discussed.

Keywords: Rheumatoid arthritis, drug, p38 MAPK.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, primarily located in the synovial joints, leading to destruction of the cartilage and bone as a result of the chronic disease activity [1]. RA affects 0.5 - 1% of the population in the industrialized world, is two to three times more frequent in women than in men and can lead to disability and reduced quality of life.

Pathogenesis

The cause of RA is still unknown, but both genetic and environmental factors play a role. The association with certain human leukocyte antigen (HLA) DR4 subtypes and RA has been recognized for a long time [2], and now it is found that rather specific amino acid sequences, the so-called shared epitope (SE), confer the highest risk for developing destructive RA [3]. RA is characterized by the presence of autoantibodies, especially rheumatoid factors and antibodies to citrullinated proteins (anti-CCP) in the majority of the patients. Recently, in a study in blood bank donors, it was demonstrated that approximately half of patients with RA had specific serologic abnormalities (rheumatoid factor and anti-CCP antibodies) several years before the onset of symptoms [4]. The earliest events in RA might involve activation of the innate immune system, which triggers a T-cell response possibly directed towards citrullinated proteins [5]. Infiltrating T-cells in the synovial membrane may, by cell-cell contact, and activation by different cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ and interleukin (IL)-17, activate monocytes, macrophages and synovial fibroblasts. These cells than produce pro-inflammatory cytokines, mainly TNF-α, IL-1 and IL-6 [6]. As the disease progresses multiple cytokine networks become established, and these cytokines trigger the production of matrix metalloproteinases (MMPs), resulting in irreversible damage of cartilage and bone [7].

Therapy

DMARDS (disease modifying anti-rheumatic drugs) are drugs that are intended to inhibit both the inflammatory and destructive processes in RA. Of these DMARDS methotrexate (MTX) is the most commonly used and regarded as the gold standard of DMARD therapy [8]. At doses used in the treatment of RA, MTX is likely to act *via* a number of intracellular pathways. Upon transport into cells, MTX is converted to polyglutamated forms, which promotes intracellular retention. This results eventually in induced release of adenosine, which has an anti-inflammatory effect on neutrophils and mononuclear cells [8,9]. MTX modulates cytokine responses at a number of levels and may promote apoptosis of activated lymphocytes [10]. Since five years a new DMARD has become available, Leflunomide, which is an active metabolite that inhibits dihydro-orotate-dehydrogenase, an enzyme involved in *de novo* pyrimidine synthesis [11]. Inhibition of this enzyme affects various signal-transduction mechanisms, the generation of cytokines, and cell proliferation and migration.

However, these DMARDS still have limited efficacy and may lead to toxicity problems. The pro-inflammatory role of cytokines and the involvement of different cell types led to the development of therapeutics to selectively target cytokines. The key role of TNF-α in the pathogenesis of RA was demonstrated both in experimental animal models and in RA patients by Feldmann, Maini and others [12]. There are now three drugs in use for treatment that block the activity of TNF-α: Infliximab (chimeric monoclonal antibody to human TNF), Adalimumab (human monoclonal antibody to TNF) and Etanercept (soluble TNF receptor construct) and one to block IL-1 activity: Anakinra (IL-1 receptor antagonist). The efficacy and possible toxic effects of these new drugs have been reviewed by Olsen and Stein [13]. Although TNF blockade has been a major breakthrough in the therapy of RA in the last ten years, there are some drawbacks. About half of the patients in clinical trials do not achieve adequate clinical responses expressed as American College of Rheumatology (ACR) 50 responses, remissions are seldom, and these drugs also have side effects, for instance increased risk of tuberculosis [13].

New drugs to inhibit the production and/or activity of inflammatory cytokines may be found in inhibitors of intracellular signal transduction pathways [14]. These pathways are involved in the primary production of these cytokines, as well as in the responses generated by these cytokines. TNF-α and IL-1 induce a variety of signal transduction cascades that

*Address correspondence to this author at the Department of Rheumatology, University Medical Center Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands; E-mail: j.westra@med.umcg.nl

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lead to the activation of transcription factors and subsequently to the expression of genes, coding for inflammatory mediators.

SIGNAL TRANSDUCTION PATHWAYS

Signal transduction pathways are intracellular mechanisms by which cells can react to extracellular stimuli such as stress and inflammatory cytokines, by converting an extracellular signal into an intracellular response. A key role is played by proteins called kinases, which act as regulators of cell function by catalyzing (facilitating) the addition of a negatively charged phosphate group to proteins. A secondary kinase is activated by phosphorylation and can activate and phosphorylate a tertiary kinase. These signaling cascades ultimately lead to induction of gene transcription and translation into specific proteins. These proteins, including cytokines, matrix metalloproteinases, and cyclo-oxygenase (COX)-2 are involved in the pathogenesis of RA.

The main signal transduction pathways implicated in RA include the mitogen-activated protein kinase (MAPK) pathways, nuclear factor-kappa B (NF-κB) and the Janus kinase / signal transducer and activator of transcription (JAK-STAT) pathway.

There are four well-characterized families of MAPKs (Fig. (**1**)), acting by phosphorylation of specific serine (Ser), threonine (Thr) and tyrosine (Tyr) residues of target substrates thereby controlling important cellular functions, such as gene expression, mitosis, movement, metabolism and apoptosis. The MAPK isoforms themselves are phosphorylated by a dual- specificity serine-threonine MAPK-kinases (MAPKK or MEK) which in turn are phosphorylated by upstream MAPK-kinase-kinases (MAPKKKs or MEKKs).

The MAPK family include the extracellular signal-regulated kinases ERK1 and ERK2 (also known as the p42/p44 MAPK pathway), the c-jun NH_2-terminal kinases JNK 1, JNK 2 and JNK 3, the four p38 enzymes, p38α, p38β, p38γ and p38δ, and the ERK5 or big MAP kinase 1 (BMK1) [15]. All MAPKs share the amino-acid sequence Thr-Xxx-Tyr in which X differs: X is glutamic acid (Glu), proline (Pro) and glycine (Gly) for the ERK, JNK and p38 MAPK respectively [16]. In general, p38 MAPKs are like the JNKs, stress-activated protein kinases, that mediate responses to cellular stress factors, such as UV light, osmotic shock and cytokines, while the ERKs are activated by proliferative and mitogenic stimuli.

p38 MAPK PATHWAY

The main activation route for p38 MAPK is through phosphorylation by MMK3 / MKK6 and possibly by MKK4, which in turn are activated by the MAPKKKs (Fig. (**2**)). Recently, it was demonstrated that MKK3 and MKK6 make individual contributions to p38 MAPK activation in rheumatoid synovial fibroblasts after cytokine stimulation, and that both must be blocked for maximal inhibition [17]. Members of the MAPKKK superfamily include MEKK 1-4, MLK, Tpl2 (tumor progression locus-2), TGFβ-activated protein kinase (TAK)-1 and apoptosis signal-regulating kinase (ASK)-1 [18]. The MAPKKKs themselves are activated by small GTP-binding proteins, including Rac and Cdc42, partly involving p21-activated kinases (PAKs). In inflammation the most important route for activation is *via* TNF-α and IL-1 by ligation of their respective extracellular membrane receptors and subsequent recruitment of intracellular adaptor molecules. Activated p38 MAPKs can phosphorylate downstream kinases (MAPK-activated protein kinase (MAPKAPK)-2, mitogen- and stress- activated protein kinase (MSK)-1, p38-regulated/activated protein kinase (PRAK) and MAPK interacting serine/threonine kinase (MNK)) and transcription factors, such as activating transcription factor (ATF)-2, CHOP (C/EBP homologous protein) and ELK-1.

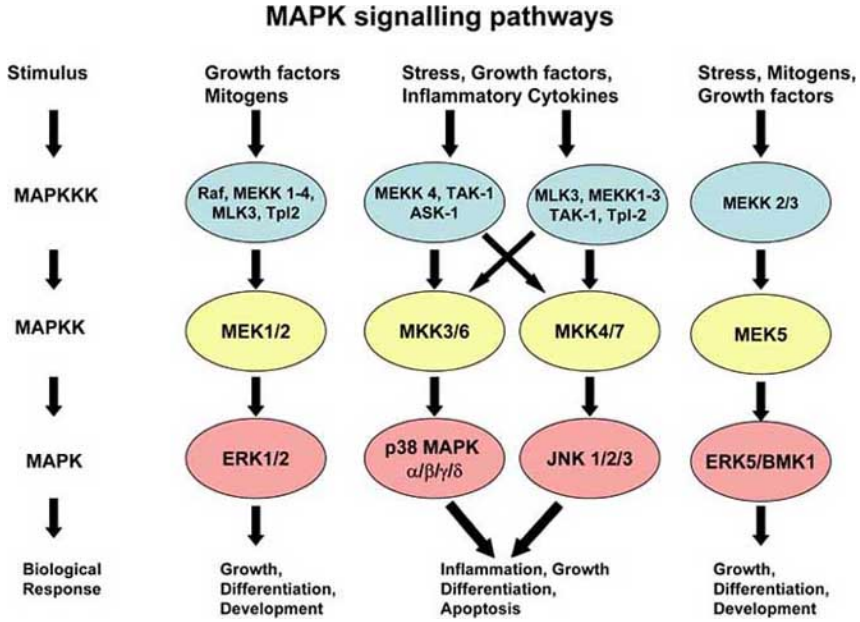

Fig. (1). Schematic presentation of MAPK signaling pathways from extracellular stimulus to biological response. See list of abbreviations.

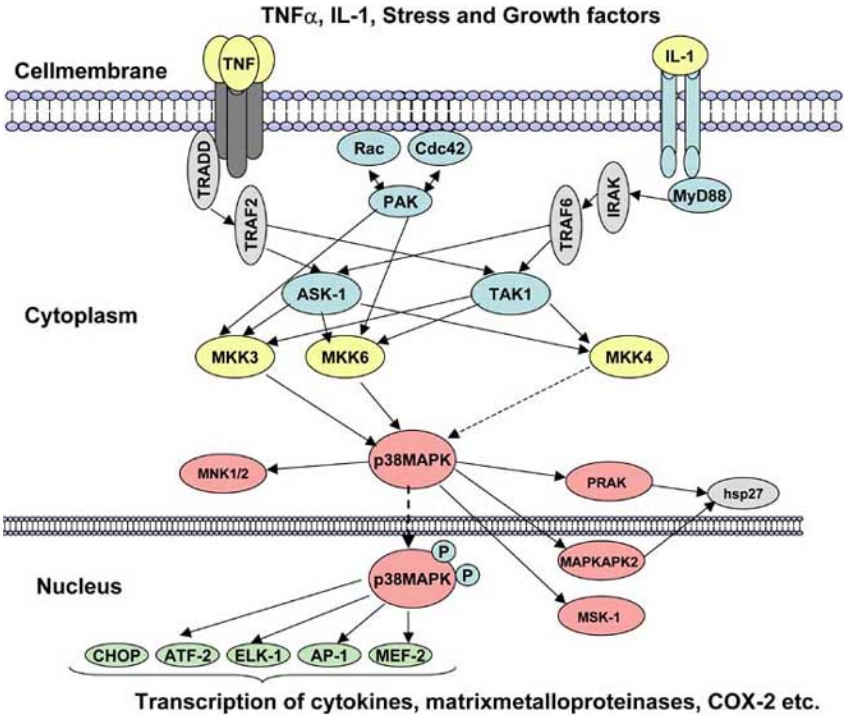

Fig. (2). Schematic presentation of p38 MAPK signal transduction pathway. The pathway is activated by the interaction of inflammatory cytokines or growth factors with their receptors, leading to phosphorylation of kinases. The result is transcription of inflammatory cytokines, matrix metalloproteinases and COX-2. See list of abbreviations.

Lipopolysaccharide (LPS) signal transduction is also one of the activation routes for p38 MAPK. LPS is a common constituent of Gram-negative bacterial outer membranes and is the principal initiator of septic shock. Signaling of LPS involves a receptor complex with Toll-like receptor 4 (TLR-4), CD14 and the adaptor molecules MyD88 and IRAK (interleukin-receptor associated kinase). The route then resembles the IL-1 route: *via* TNF-receptor associated factor (TRAF)6 and TAK1 p38 MAPK as well as the JNK and the NF-κB pathways can be activated [19].

Other activation mechanism for p38α MAPK have been described that do not involve the prototypic kinase cascade. The first mechanism depends on interaction of p38α with TAB1 [(TAK1)-binding protein 1] leading to autophosphorylation and activation of p38α MAPK [20]. In this study by Ge *et al.* the formation of a TRAF6-TAB1-p38α complex was detected and activation of stimulus-specific TAB1-dependent and TAB1-independent p38α MAPK was shown. An other activating mechanism described showed that T cell receptor (TCR) ligation increased $p56^{lck}$ activity and upregulation of Zap70 activity, followed by phosphorylation of Tyr323 of p38 MAPK and the subsequent autophosphorylation of Thr180-Tyr182 and activation of the kinase [21]. The latter activation pathway is not stressful in T-cells and these findings suggest that alternative activation pathways contribute to the biological responses of p38α MAPK to various stimuli.

IDENTIFICATION

The human p38α MAPK were first identified as the molecular target of pyridinyl imidazole class of compounds, that were known to block TNF-α and IL-1 release from LPS-stimulated human monocytes [22]. Originally these proteins were designated cytokine-suppressive anti-inflammatory drug-binding proteins (CSBP) [23]. Until now, five isoforms of p38 MAPK have been identified: p38β1 and p38β2 have more than 70% identity to p38α, whereas p38γ and p38δ have approximately 60% identity to p38α. Functional differences between the isoforms are related to their differential expression, activation, and substrate specificity [24]. p38α, p38β and p38δ are widely produced in various tissues, while p38γ is expressed primarily in skeletal muscle. Inflammatory cells synthesize predominantly p38α and p38δ protein, but endothelial cells also produce p38β [25,26].

ERK, JNK and p38 MAPK activation were almost exclusively found in synovial tissue from RA, but not osteoarthritis patients. p38 MAPK activation was observed in the synovial lining layer and in synovial endothelial cells [27].

DOWNSTREAM EFFECTS OF P38 MAPK ACTIVATION

Gene Expression

One of the main downstream effects of the p38 MAPK pathway is regulation of gene expression, which can be at the transcriptional level, but also at the translational level, leading to protein synthesis. p38 MAPK has been implicated in the activation of various transcription factors including: ATF-2, myocyte enhancer factor (MEF)-2C, CHOP, and NF-κB [28-30]. Other transcription factors are phosphorylated by downstream protein kinases that are themselves activated by phosphorylated p38 MAPK, such as MAPKAPK-2. Fur-

thermore MAPKAPK-2 deficient mice show diminished production of IL-6 and TNF-α [31]. These effects are thought to be mediated by a mechanism involving mRNA turnover and protein translation. The p38 MAPK/MAPKAPK-2 pathway is crucial to inflammatory cytokine production [32;33] but also to the expression of MMPs in chondrocytes [34] and in synovial fibroblasts [35].

Post Transcriptional Regulation of mRNA Stability

As will be discussed later, several p38 MAPK inhibitors have been developed which were shown to block the production of inflammatory cytokines. This inhibition seems to be a combined effect at the level of transcription and translation. Inducible cytokines usually have short lived mRNAs and contain an AU-rich element (ARE) in their 3'untranslated region responsible for their high turn-over rate [36]. These AREs contain repeats of the motif AUUUA, and were discovered as instability elements. In the case of inflammatory response mRNAs, the instability is countered by signaling in the p38 MAPK pathway [37]. Under normal conditions, these AU-rich elements are occupied by AU-binding proteins, thereby blocking translation or transcription. It has been demonstrated that upon stimulation these AU-binding proteins are phosphorylated in a p38 MAPK-dependent manner, resulting in their dissociation, and allowing translation of these mRNAs [37,38]. Inhibitors of p38 MAPK can target these events directly or via MAPKAPK-2. It was shown that both translation and stability of TNF-α mRNA are regulated by the p38MAPK pathway [39], IL-6 and IL-8 mRNA stability is only regulated by p38 MAPK. The extent of inhibition of resulting protein production varies with cell type [40,41]. Activation of p38 MAPK has also been shown to enhance the mRNA stability of collagenase-1 (MMP-1) and stromelysin-1 (MMP-3) [42]. Finally, Lasa *et al.* demonstrated that the glucocorticoid dexamethasone destabilizes COX-2 mRNA by inhibiting p38 MAPK. This effect was induced by expression of MAPK-phosphatase-1 (MKP-1), which inhibits the MAPK activation by specific dephosphorylation [43].

Role in Regulation of NF-κB Recruitment

Association of histone proteins with DNA is essential to the regulation of gene expression. The NH_2 – termini of the four core histones (H2A, H2B, H3 and H4) protrude out of the nucleosome and undergo different covalent modifications – including acetylation, phosphorylation and methylation – that affect chromatin structure and, either directly or indirectly, transcription [44]. Saccani *et al.* found that inflammatory stimuli induce p38 MAPK-dependent phosphorylation and phosphoacetylation of histone H3; this selectively occurred on the promoters of a subset of stimulus-induced cytokine and chemokine genes [45]. p38 MAPK activity was required to enhance the accessibility of the cryptic NF-κB binding sites contained in H3 phosphorylated promoters, which indicated that p38-dependent H3 phosphorylation may mark promoters for increased NF-κB recruitment. These results show that p38 MAPK plays an additional role in the induction of the inflammatory and immune response: the regulation of NF-κB recruitment to selected chromatin targets.

Inactivation by Phosphatases

For control of the MAPK signal transduction pathways dephosphorylation is necessary. Over the last decade a family of endogenous negative regulators of MAPK, the MAPK phosphatases (MKPs), also known as DUSPs (dual-specificity phosphatases) have been described [46]. The MKPs dephosphorylate the tyrosine and threonine motifs of MAPK to deactivate MAPK-dependent signaling. At least 10 mammalian MKPs have been cloned and characterized, which have different subcellular distribution, substrate specificity, and expression patterns. MKP-1 was the first isolated MKP, and is a 39,5-kd protein that is preferentially localized in the cell nucleus. It is capable of dephosphorylating all MAPK families, although a preference for dephosphorylating p38 MAPK and JNK has been described [47]. MKP-1 is an early response gene that is induced by the same stimuli that activate the MAPKs, such as cytokines, osmotic shock and UV radiation [48,49]. Recently, the expression of MKP-1 in rheumatoid synovial fibroblasts was demonstrated as well as a role for glucocorticoid dependent upregulation of MKP-1[48].

p38 MAPK INHIBITORS

Development

In 1988 the inhibition of IL-1 production by the anti-inflammatory compound SK&F 86002 was reported [50], but it took until 1994 to discover that the molecular target of the pyridinyl imidazole class of compounds indeed proved to be p38 MAPK, originally known as CSBP [22]. Since the original report of the efficacy of these compounds, they have become the most widely studied inhibitors of this kinase. The compounds have been used as framework for further synthetic work and have been utilized to elucidate the role of p38 MAPK in the immune system. Crystallographic and kinetic experiments have shown that the pyridinyl imidazole family of p38 MAPK inhibitors bind at the ATP binding site of p38 MAPK and compete with ATP for binding to active, phosphorylated p38 MAPK [51]. A common feature observed in all crystal structures of p38 MAPK in complex with pyridine-imidazole derivatives is the formation of a hydrogen bond between the backbone NH of Met109 in the linker region and the pyridine nitrogen of the inhibitor [52].

After the structure-activity relationship was established SB 203580 and other 2, 4, 5- triaryl imidazoles were prepared as pharmacological tools to regulate cytokine synthesis. A large number of preclinical studies have been reported on specific and selective p38 MAPK inhibitors that block the production of inflammatory cytokines *in vitro* and *in vivo* [53], as well as inhibition of several other inflammatory molecules such as COX-2 and inducible nitric oxide synthase (iNOS).

The clinical development of p38 MAPK inhibitors has been obstructed by liver toxicity, due to interference with hepatic cytochrome P450 enzymes [54]. By replacing substituents at the pyridinyl and imidazole moiety efficacy could be maintained and toxicity reduced [52].

Boehringer developed a novel type of p38 MAPK inhibitor, an N-pyrazole-N'-naphthyl urea compound, which could bind to the kinase with slow association and dissociation rate

[55]. This compound exploited novel binding sites within the ATP pocket and inhibits p38 MAPK by inducing a conformational change that is incompatible with ATP binding.

p38 MAPK Inhibitors in Animal Models for Arthritis

Several p38 MAPK inhibitors have been evaluated in animal arthritis models. The most relevant compounds are listed in Table **1A** and structure formulas are shown in Fig. (**3**). In 1996 the pharmacological profile of SB 203580 (GlaxoSmithKline) was investigated in adjuvant-induced arthritis in Lewis rats and murine collagen induced arthritis [56], whereas in 1998 the pharmacologically effects of SB 220025 (GlaxoSmithKline) were investigated in a model of inflammatory angiogenesis is the murine air pouch granuloma, which has a hyperangiogenic component [57]. RPR-200765A (Aventis) reduced incidence and progression in the rat streptococcal cell wall (SCW) arthritis model at doses given orally [58], while prevention of the onset and progression of collagen-induced arthritis were reported for FR167653 (Fujisawa Pharmaceuticals) [59]. Preclinical pharmacokinetics of SB 242235 (GlaxoSmithKline) were determined in different species [60] and recently SB 242235 was evaluated in a new model of arthritis, pristane-induced arthritis, and demonstrated to reduce significantly all arthritis scores [61]. R-130823, a p38 MAPK inhibitor developed by Sankyo, was reported to ameliorate hyperalgesia and swelling in animal arthritis models [62]. Other pharmaceutical companies have reported development and effects of different p38 MAPK inhibitors as well : Novartis [63], Bristol Meyers Squibb [64], and Eli Lilly [65].

p38 MAPK Inhibitors in Human Studies

Until now very little studies on p38 MAPK inhibition in human studies have been published (see Table **1B** and Fig. (**4**)). Single-dose pharmacokinetics and pharmacodynamics of a Johnson and Johnson compound, RWJ 67657, were investigated in healthy male subjects [66]. RWJ 67657 was rapidly absorbed (mean tmax = 0.6-2.5 h) and there were no significant adverse effects associated with single doses of this drug. The effects of this compound on clinical and cytokine response to endotoxemia were studied in healthy human volunteers and reported by Fijen *et al*. [67]. Single oral doses of RWJ 67657 dose-dependently decreased symptoms and elevated cytokine levels, induced after administration of endotoxin. Inhibition of coagulation, fibrinolysis and endothelial cell activation by BIRB-796 during human endotoxemia was reported by Branger *et al*. [68]. Doramapimod (BIRB-796) is in phase III trials for psoriasis and phase IIa trials for rheumatoid arthritis and Crohn's disease (Current Drug Discovery, February 2004). Doramapimod is also being investigated for the potential treatment of endotoxic shock. With semapimod (CNI-1493), a tetravalent guanylhy-

Table 1.

compound	company	model	references
A. p38 MAPK inhibitors in animal arthritis studies			
SB 203580	GlaxoSmithKline	AA rat, CIA mice	[56]
SB220025	GlaxoSmithKline	air pouch granuloma mice	[57]
RPR-200765A	Aventis	streptococcal wall induced A rats	[58]
FR167653	Fujisawa Pharma	CIA	[59]
SB242235	GlaxoSmithKline	pharmacokinetics	[60]
SB242235	GlaxoSmithKline	pristane induced arthritis	[61]
R-130823	Sankyo	AA	[62]
B. p38 MAPK inhibitors in human studies			
RWJ67657	Johnson & Johnson	pharmacokinetics/-dynamics	[66]
RWJ67657	Johnson & Johnson	endotoxemia	[67]
BIRB-796	Boehringer Ingelheim	endotoxemia	[68]
BIRB-796	Boehringer Ingelheim	phase III psoriasis	
BIRB-796	Boehringer Ingelheim	phase IIA RA	
CNI-1493 *	Cytokine Pharma	phase III Crohn's disease	[69]
VX-745	Vertex	phase II RA	[70]
VX-702	Vertex	phase II various inflam.conditions	[71]
SCIO-469	Scios Inc	phase II RA	[72]

*(also JNK inhibitor)

AA-adjuvant induced arthritis, CIA-collagen induced arthritis

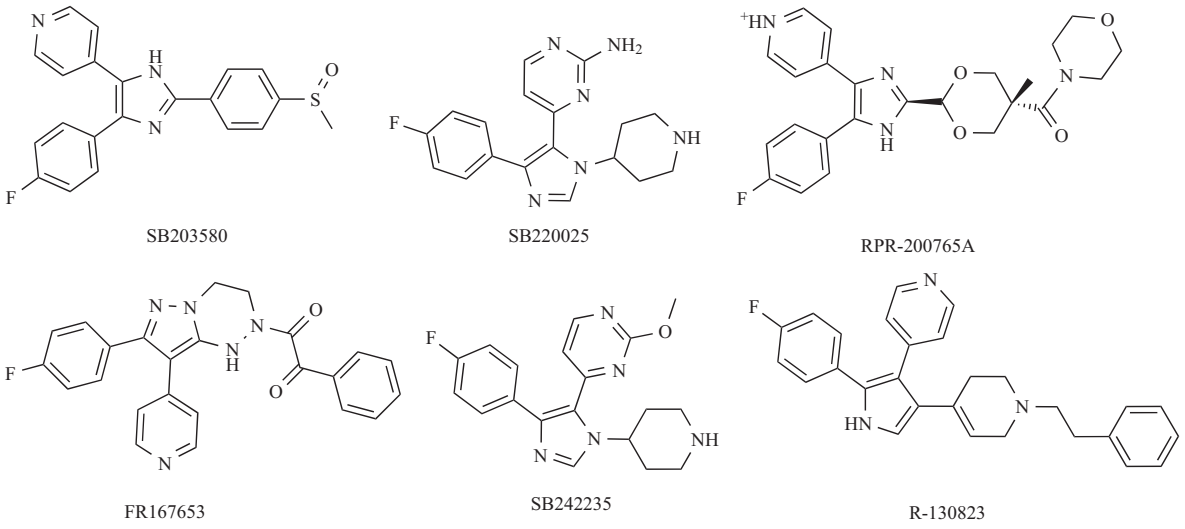

Fig. (3). Structure formulas of p38 MAPK inhibitors used in animal arthritis studies.

drazone compound, which inhibits both JNK and p38 MAPK, clinical improvement was induced in patients with moderate to severe Crohn's disease [69].

Vertex has investigated a number of compounds. VX-745 has been in phase II trials for rheumatoid arthritis [70], but has been withdrawn due to side effects such as elevated liver enzymes and central nervous system toxicity. The follow-up compound VX-702 (structure formula not revealed), that does not cross the blood-brain barrier, has now reached phase II for various inflammatory conditions, for instance in patients with acute coronary syndrome [71] and also for RA patients according to press releases (2005) from Vertex. One of the most promising p38 MAPK inhibitors for treatment of rheumatoid arthritis is SCIO-469, which is now in phase II clinical trial [72]. Scios has not revealed the structure formula but suggested that may be it is an indole-piperazine-based compound.

CONCLUSION

The discovery of p38 MAPK inhibitors has dramatically increased the understanding of signal transduction pathways involved in inflammation. It is widely expected that p38

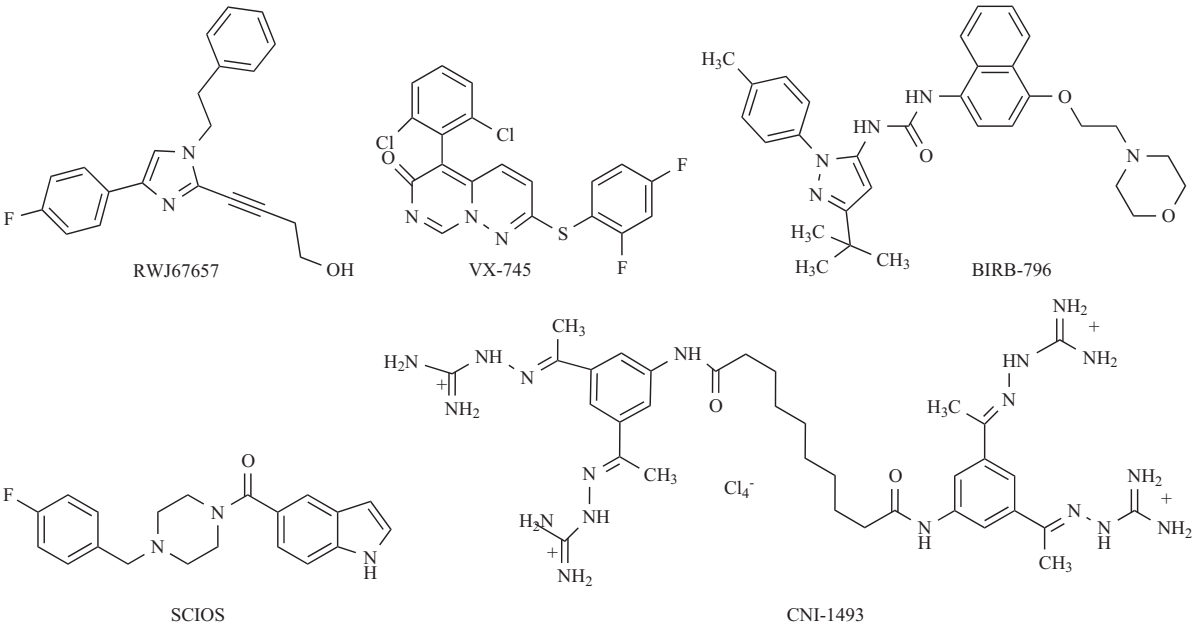

Fig. (4). Structure formulas of p38 MAPK inhibitors used in human studies.

MAPK inhibitors will have efficacy in arthritis and other inflammatory diseases.

Prous Science's Integrity® drug discovery portal contains structures of 955 compounds that have been mentioned in current literature and/or described in patent literature as p38 MAPK inhibitors [73]. In the years results of clinical trials will be published, revealing whether p38 MAPK inhibitors will be the next breakthrough in therapy for RA.

ABBREVIATIONS

AP-1 = activating protein kinase

ASK = apoptosis signal-regulating kinase

ATF = activating transcription factor

BMK = big MAP kinase

CHOP = C/EBP homologous protein

C/EBP = CCAAt/enhancer binding protein

COX = cyclo-oxygenase

CREB = cyclic AMP-responsive element-binding protein

ERK = extracellular signal-regulated kinase

Hsp = heat shock protein

IκB = inhibitor of NF-κB

IKK = IκB kinase

IL = interleukin

iNOS = inducible nitro-oxide synthase

IRAK = interleukin related associated kinase

JAK = janus kinase

JNK = c-Jun NH_2 -terminal kinase

MAPK = mitogen activated-protein kinase

MAPKK = MAPK kinase kinase

MAPKKK = MAPK kinase kinase kinase

MAPKAPK = MAPK activated protein kinase

MEF = myocyte enhancer factor

MEK = MAPK or ERK kinase

MEKK = MEK kinase

MKK = MAPK kinase

MLK = mixed lineage kinase

MNK = MAPK interacting serine/threonine kinase

MSK = mitogen- and stress- activated protein kinase

NAK = NF-κB activating kinase

NIK = NF-κB inducing kinase

NF-κB = nuclear factor immunoglobulin κ chain enhancer B-cell

PAK = p21-activated kinase

PRAK = p38-regulated/activated protein kinase

STAT = signal transducer and activator of transcription

SHP = SH2-domain containing tyrosine phosphatase

TAK = TGFβ-activated protein kinase

TGFβ = transforming growth factor β

TNF = tumor necrosis factor

Tpl-2 = tumor progression locus-2

TRADD = TNF-receptor-1 associated death domain

TRAF = TNF-receptor associated factor

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