Review

MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits

Bozena Kaminska*

Department of Cell Biology, Laboratory of Transcription Regulation, Nencki Institute of Experimental Biology, 3 Pasteur Str., 02-093 Warsaw, Poland

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Abstract

Excessive inflammation is becoming accepted as a critical factor in many human diseases, including inflammatory and autoimmune disorders, neurodegenerative conditions, infection, cardiovascular diseases, and cancer. Cerebral ischemia and neurodegenerative diseases are accompanied by a marked inflammatory reaction that is initiated by expression of cytokines, adhesion molecules, and other inflammatory mediators, including prostanoids and nitric oxide. This review discusses recent advances regarding the detrimental effects of inflammation, the regulation of inflammatory signalling pathways in various diseases, and the potential molecular targets for anti-inflammatory therapy. Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases that mediate fundamental biological processes and cellular responses to external stress signals. Increased activity of MAPK, in particular p38 MAPK, and their involvement in the regulation of the synthesis of inflammation mediators at the level of transcription and translation, make them potential targets for anti-inflammatory therapeutics. Inhibitors targeting p38 MAPK and JNK pathways have been developed, and preclinical data suggest that they exhibit anti-inflammatory activity. This review discusses how these novel drugs modulate the activity of the p38 MAPK and JNK signalling cascades, and exhibit anti-inflammatory effects in preclinical disease models, primarily through the inhibition of the expression of inflammatory mediators. Use of MAPK inhibitors emerges as an attractive strategy because they are capable of reducing both the synthesis of pro-inflammatory cytokines and their signalling. Moreover, many of these drugs are small molecules that can be administered orally, and initial results of clinical trials have shown clinical benefits in patients with chronic inflammatory disease.

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1. Introduction

Inflammation, a key process in the host defence system, is highly regulated in order to restrict its action to the time and place where it is necessary. Loss of control can lead to a number of diseases, including rheumatoid arthritis, chronic inflammatory bowel diseases, neurodegenerative disorders, and septic shock syndrome [1,2]. Triggered by a variety of physical, chemical, or biological agents, inflammation is a cumulative result of genetic susceptibility factors and multiple responses, both paracrine and autocrine in nature. Although the inflammatory responses are different in various diseases, they can be characterised by a common spectrum of genes and endogenous mediators involved, including growth factors, inflammatory cytokines such as interleukin 1 β (IL-1β), tumour necrosis factor (TNF)-α, interleukin-6 (IL-6), chemokines (Macrophage Inflammatory Factor—MIP-1α, IL-8), matrix metalloproteinases, and toxic molecules such as nitric oxide or free radicals [3,4].

Rheumatoid arthritis is a chronic and systemic disorder that is characterised by progressive destruction of articular cartilage and bone. The aetiology of rheumatoid arthritis has still not been elucidated but it is thought to be triggered by a combination of genetic susceptibility and exposure to environ-
mental factors. TNF-α, IL-1β, -6, and -8 are significantly abundant in joint lesions of rheumatoid arthritis patients. These cytokines are mostly released from the macrophages that infiltrate joint lesions (for review, see [5,6]). Monoclonal antibodies against TNF-α or TNF-α receptor-Fc fusion protein suppress the symptoms and disease progress of rheumatoid arthritis [7–9]. Similarly, patients treated with the IL-1 receptor antagonist showed improvement in clinical studies [10].

Inflammatory bowel disease encompasses a number of chronic, relapsing inflammatory disorders involving the gastrointestinal tract. Ulcerative colitis and Crohn’s disease are two entities of chronic inflammatory bowel diseases in which a dysregulated immune response triggered by products of the enteric bacterial flora, viral and, perhaps, dietary antigens, is one of the main pathogenic mechanisms [11]. TNF plays a central role in the initiation and amplification of the inflammatory reaction in Crohn’s disease [12]. Monoclonal antibodies against TNF have proven clinically effective [13].

Tissue damage in acute and chronic neurodegenerative diseases is a result of a complex pathophysiological cascade which comprises a variety of distinct pathological events [14]. One of the common pathophysiological hallmarks of neurodegenerative disorders, such as Alzheimer’s disease, Parkinson’s disease, HIV-associated dementia, brain trauma, and stroke, is microglial activation [15,16]. Resident non-neuronal brain cells respond rapidly to neuronal cell death and may have both deleterious and beneficial roles in neuronal damage. Microglial cells activated in vitro produce toxic reactive oxygen radicals and NO, substances that may directly damage neurons. Activated microglia release neurotoxic and pro-inflammatory cytokines such as FasL, TNF-α, IL-1, IL-6, INF-γ, and numerous chemokines [17–20]. Cytokines produced by microglia may further activate astrocytes that in turn become a source of neurotoxic cytokines. Growing evidence indicates that the inhibition of secretion or activity of IL-1β and TNF-α with neutralising antibodies or soluble cytokine receptors leads to a decrease in neuronal damage [21,22]. Knockout mice deficient in neuronal nitric oxide synthase [23], or IL-1β converting enzyme—ICE [21] are less vulnerable to toxic insult. The role of TNF-α in brain ischemia is controversial and probably depends on experimental conditions [24]. TNF-α is one of the mediators dramatically increased after brain injury that leads to the activation, proliferation, and hypertrophy of phagocytic cells and gliosis. Its inhibition by pharmacological agents, neutralising antibodies or soluble cytokine receptors has protective effects. In contrast, reports from in vitro studies and knock-out mice suggest rather beneficial effects of TNF-α (for a review see [22]).

Astrocytes, the major glial cell type in the brain, are important contributors to inflammatory immune responses within the brain. Astrocyte hypertrophy and proliferation (called reactive gliosis) is a widespread response to damage of neurons. Astrocytes produce several neurotrophic substances that regulate viability of neurons after ischemia, but they are also a source of pro-inflammatory (IL-1, IL-6) and cytotoxic cytokines (FasL, TNF-α, TGF-β). Activated astrocytes also produce toxic molecules such as reactive oxygen species and NO (for a review, see [25]).

2. MAPK involvement in regulation of crucial inflammation mediators

2.1. Activation of p38 MAPK signal transduction pathway during inflammation

In response to inflammatory stimuli that activate macrophages, intracellular signalling pathways are activated that carry the signal needed to activate the production of inflammatory mediators. Primary inflammatory stimuli (microbial products) and cytokines such as IL-1β and TNF-α, act through the Toll receptors, IL-1 receptor (TIR) family or the TNF receptor family, respectively. Activation of receptors triggers major intracellular signalling pathways: mitogen-activated protein kinase (MAPK) pathways [26–29], and the pathway leading to activation of the transcription factor nuclear factor kappa B (NF-κB) [26,30]. TNF-α is a potent activator of NF-κB, which in turn is a potent inducer of TNF-α. This positive feedback is key to chronic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. Lipopolysaccharide (LPS), a component of bacterial wall and commonly used inducer of the monocyte/macrophage cell lineage, acting via Toll-like receptor 4 (TLR4), also stimulates mitogen-activated protein (MAP) kinase cascades and the pathway leading to activation of NF-κB.

Three major groups of distinctly regulated MAP kinase cascades are known in humans that lead to altered gene expression: ERK1/2, JNK, and p38 MAP kinase. ERK are activated by MAP kinase kinase (MKK) and MKK2, JNK by MKK4 and MKK7, and p38 MAP kinase by MKK3, MKK4, and MKK6 [31,32] (Fig. 1). Upon activation of the MAP kinases, transcription factors present in the cytoplasm or nucleus are phosphorylated and activated, leading to expression of target genes resulting in a biological response. It has been demonstrated that MAP kinases have overlapping substrate specificities and phosphorylation of regulatory sites is shared among multiple protein kinases. The multiple interactions between the different MAP kinase cascades serve to integrate the responses and activate separate sets of genes [28,31].

The p38 MAP kinase pathway shares many similarities with the other MAP kinase cascades, being associated with inflammation, cell growth, cell differentiation, and cell death [28]. To date, four p38 MAP kinase isoforms have been identified sharing about 60% homology. Two isoforms (p38α, and p38β) are ubiquitously expressed, p38γ is predominantly expressed in skeletal muscle, whereas p38δ gene expression is found in the lung, kidney, testis, pancreas, and small intestine. p38 MAP is activated by dual phosphorylation on Thr180 and Tyr182 by upstream MAP kinases: MAP2K6 or MAP2K3 (MKK3/6), which are activated by upstream MAPKKKs, and stimulated by a variety of stimuli [31,32]. A MAPKK-dependent mechanism of p38 activation involves TAB1 (transforming growth factor-β-activated protein kinase 1 (TAK1)-binding protein 1).

Extracellular stimuli of the p38 MAP kinase pathway include a variety of cytokines (IL-1α, IL-2, IL-7, IL-17, IL-
18, TGF-β, and TNF-α) [31]. A number of pathogenic stimuli, including LPS, staphylococcal peptidoglycan and enterotoxin B, echovirus 1 and herpes simplex virus 1, activate p38 through different Toll receptors [33]. In most inflammatory cells, p38α is the major isoform of p38 MAPK, activated and crucial to inflammatory cytokine production and signalling [27,29].

Pyridinylimidazole compounds, exemplified by SB 203580 (Fig. 2), originally were developed as inhibitors of inflammatory cytokine synthesis [34,35]. Subsequently, the compounds were found to be selective inhibitors of p38 MAPK, inhibiting the catalytic activity of the kinase by competitive binding in the ATP pocket [36]. SB 203580 is known to inhibit the p38α and β isoforms. This selectivity is due to the presence of threonine at position 106 in p38α and β but also involves His107 and Leu108 [37]. Additional data suggest that this class of compounds bind equally well to the activated (phosphorylated on Thr180 and Tyr182) and non-activated p38 enzyme, whereas ATP binds only to the activated form [38].

2.2. Involvement of p38 MAPK in the regulation of pro-inflammatory cytokine expression

The main biological response of p38 activation involves the production and activation of inflammatory mediators to initiate leukocyte recruitment and activation. p38 MAPK positively regulates expression of many genes involved in inflammation, such as those coding for TNF-α, IL-1β, IL-6, IL-8, cyclooxygenase-2, and collagenase-1, -3 [28,39,40,41,42,43,44]. Inhibition of p38 MAPK with SB203580 reduced pro-inflammatory cytokine production in monocyte/macrophages, neutrophiles, and T lymphocytes. The expression of pro-inflammatory cytokines can be regulated at both the transcriptional and post-transcriptional levels.

2.2.1. Involvement of p38 MAPK in transcriptional regulation of pro-inflammatory cytokine expression

Transcriptional activation of the TNF-α gene in murine macrophages has been demonstrated to be predominantly dependent on a region in the murine TNF-α promoter upstream of −451 bp, which contains NF-κB binding motifs [45]. However, further studies have defined two cis-acting regulatory elements in the human TNF-α promoter that mediate its maximal expression in response to LPS: region I contained an overlapping Sp1/Egr-1 site, whereas region II contains CRE and NF-κB sites. LPS stimulation increases the binding of c-Jun-containing complexes to the CRE sites [46]. It is believed that cooperation between different inducible transcription factors, such as NF-κB, Egr1, and c-Jun/AP-1, is responsible for full induction of the TNF-α promoter [46–48].

p38 MAPK can modulate transcriptional up-regulation of TNF-α expression in many different ways. p38 MAPK can increase the expression, or regulate by phosphorylation, several transcription factors, including activating transcription factor-2 (ATF-2 is a possible partner of c-Jun in the AP-1 complex), GADD153, myocyte enhancer factor 2C (MEF 2C), C/EBP homologous protein 1 [31,49,50], and NF-κB [51]. The mechanisms by which the p38 MAP kinase regulates NF-κB-dependent gene expression in LPS-stimulated macrophages are complex. Carter et al. [52] found that NF-κB-dependent gene expression was reduced by SB 203580 or a dominant-negative p38 MAP kinase vector, however, such treatment did not alter NF-κB activation or DNA binding, which suggested indirect influence on expression. Subunits of NF-κB are known to interact with basal transcription factors, such as TFIIB, and TATA-binding protein (TBP). Since inhibition of the p38 MAP kinase significantly reduced the DNA binding of TBP to the TATA box, it is possible that the p38 MAP kinase regulates NF-κB-dependent transcription in part by modulating activation of basal transcription factors [52,53].

2.2.2. Involvement of p38 MAPK in post-transcriptional regulation of pro-inflammatory cytokine expression

Many of the mRNAs coding for inflammatory response genes are unstable and they are stabilised by the p38 MAPK pathway [54,55]. The presence of AU-rich sequences in the 3′-untranslated region (3′-UTR) of a number of inflammatory mediator genes and immediate early genes is critical for their post-transcriptional regulation [56,57]. These sequences are
characterised by the presence of one or more copies of AUUUA, and bind proteins involved in stabilising these mRNAs [57]. In the case of TNF-α, the AU-rich element (ARE) is also known to be involved in repressing the translation of this mRNA [58,59], while in the case of IL-6, it is critical for mRNA stability [59]. Control of mRNA stability is a mechanism of increasing the amplitude of the response and allows rapid adjustment of mRNA levels.

Activation of p38 MAP kinase, triggered by the inflammatory cytokine IL-1β or by bacterial lipopolysaccharide, induces stabilisation of ARE-containing endogenous mRNAs and reporter mRNAs containing several different AREs, including those of IL-3, IL-6, IL-8, TNF-α, cyclooxygenase 2, and c-fos. The effect of p38 MAPK on TNF-α synthesis is mainly mediated via the activation of its downstream target MAPKAP-K2, because the LPS-induced production of TNF-α in vivo is decreased by 90% in mice that do not express MAPKAP-K2 [59,60]. The production of IL-6 and interferon-γ is reduced similarly in MAPKAP-K2-deficient mice [60].

Recent studies demonstrated that in macrophages p38 MAPK regulates TNF-α expression by a dual mechanism, in part by the 3′ UTR as previously suggested, but also via the 5′ promoter region, putatively through NF-κB [53]. Inhibition of p38 MAPK results in a reduction of TNF-α mRNA in human macrophages, and this effect is partially mediated by inhibition of the 5′ promoter region of the TNF-α gene. Studies with pharmacological inhibitors or adenovirally mediated overexpression of a dominant negative p38 MAPK demonstrated that the kinase is involved in modulating the transactivating function of NF-κB in LPS-treated macrophages [53].

IL-8 induction is an example of highly inducible gene expression in response to IL-1β, and formation of large amounts of this cytokine requires the co-ordinated activation of several pathways. IL-8 gene regulation is under complex transcriptional control, involving AP-1, CAAT/enhancer-binding protein, and NF-κB binding sites, as well as under post-transcriptional control mediated by AU-rich mRNA regions [61]. Overexpression of active forms of NIK (NF-κB-inducing kinase), MKK7, and MKK6 is sufficient to induce IL-8 expression. Further studies demonstrated that IL-8 transcription is controlled by the co-ordinated action of the NF-κB and JNK pathways, whereas IL-8 mRNA degradation is regulated by the p38 MAPK pathway [61]. TAK1 appears to be an upstream kinase responsible for the activation of JNK and p38.
MAPK in the regulation of IL-8 formation in response to IL-1β [62].

The p38 MAPK signalling pathway regulates Interleukin-6 gene expression and synthesis in response to IL-1β and TNF-α [63,64].

2.3. Involvement of p38 MAPK in the regulation of an inducible NO synthase

Stimulation of macrophages by LPS results in the expression of an inducible NO synthase (iNOS) which catalyses the production of large amounts of NO from L-arginine and molecular oxygen. NO, in turn, participates in the inflammatory response of macrophages [65]. p38 MAPK activation is involved in iNOS expression in tumour necrosis factor-α and interleukin-1β-stimulated mouse astrocytes [66], as well as in LPS-stimulated mouse macrophages [67,68]. The promoter of the murine gene encoding iNOS contains two NFκB-binding sites, located at 55 and 971 base pairs upstream of the TATA box, respectively [69], and protein binding to the NFκB sites is necessary to confer inducibility of iNOS by LPS [70].

3. Inhibitors of MAPK signalling pathways as anti-inflammatory drugs

3.1. Inhibitors of p38 MAPK as anti-inflammatory drugs in rheumatoid arthritis

Multiple signal transduction pathways, including MAPK signalling, have been implicated in rheumatoid arthritis [5,6], and preclinical models have confirmed the therapeutic potential of small-molecule inhibitors. SB 203580 and SB 220025, both inhibitors of p38 MAPK and cytokine synthesis, were effective in a murine model of collagen-induced arthritis [71]. SB 242235, another member of the pyrindyl imidazole class, potently inhibits TNF-α and has protective effects in antigen-induced arthritis [72].

Wada et al. [73] described the anti-inflammatory effects of the synthetic compound R-130823 {2-(4-fluorophenyl)-4-(1-phenethyl-1,2,3,6-tetrahydropyridin-4-yl)-3-(pyridin-4-yl)-1H-pyrrole}, which showed an inhibitory effect on p38α, and a much weaker one on p38β. R-130823 suppressed TNF-α, IL-1β, IL-6, and IL-8 release from LPS-stimulated human blood. It is noteworthy that the IC₅₀ values of R-130823 on cytokine production were 37-fold lower than the IC₅₀ values of a reference p38 MAPK inhibitor, SB 203580. Besides the anti-inflammatory and analgesic effects, R-130823 suppressed the exacerbation of murine collagen-induced arthritis by reducing hind paw swelling. Histological analysis of knee joints showed that proliferation of fibroblasts and synoviocytes, and infiltration of neutrophils, was ameliorated [73].

Recently, a library of trisubstituted oxazoles, thiazoles, imidazoles (1,2,4- and 2,4,5-substituted) and imidazo[1,2]pyridines was prepared at Novartis and such drugs were evaluated in vitro as p38α inhibitors and in vivo in animal models of rheumatoid arthritis. Four structures were identified as potent inhibitors of p38α with high efficacy in blocking the LPS-induced TNF-α release in the mouse, and decreasing adjuvant- and collagen-induced arthritis in the rat [74]. At AstraZeneca, a novel structural class of p38 MAPK inhibitors has been discovered through selectivity screening. Inhibition of human p38α enzyme activity in vitro and decrease of LPS-induced TNF-α release have been reported [75].

3.2. Inhibitors of p38 MAPK in systemic inflammation

p38 MAPK inhibitors (e.g. SB 203580) have been found to reduce LPS-induced TNF-α production in mice and rats [34], and reduced mortality in LPS-treated mice [34]. Similar effects were observed with another p38 MAPK inhibitor, RWJ 67657 [4-4-(4-fluorophenyl)-1-(3-phenylpropyl)-5-(4-pyridinyl)-1H-imidazol-2-yl]-3-butyln-1-ol (Fig. 2), which inhibited the release of TNF-α by LPS-treated human peripheral blood mononuclear cells in vitro, and TNF-α production in LPS-injected mice and rats [76].

Recent studies in humans demonstrated that the orally administered novel inhibitor of p38 MAPK, BIRB 796 BS, significantly inhibited LPS-induced p38 MAPK activation in the leukocyte fraction of volunteers, as well as cytokine production (TNF-α, IL-6, IL-10, and IL-1R antagonist). In addition, p38 MAPK inhibition diminished leukocyte responses, release of elastase-α1-antitrypsin complexes and C-reactive protein, and up-regulation of CD11b with down-regulation of L-selectin [77]. BIRB 796 BS is 1-(5-tert-butyl-2-p-tolyl)-2H-pyrrozol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)-naphthalen-1-yl]-urea, a water-soluble, orally bioavailable molecule developed by Boehringer Ingelheim Pharmaceuticals. In contrast to other p38 MAPK inhibitors (e.g., SB203580), BIRB 796 BS prevents both the phosphorylation and activity of p38 MAPK by binding to a novel allosteric binding site as well as to the ATP pocket of p38 MAPK.

3.3. Inhibitors of p38 MAPK in chronic bowel diseases

As mentioned in Section 1, two important intracellular signalling pathways have been identified in the pathogenesis of chronic inflammatory bowel diseases: the NF-κB and MAPK cascade pathways. Immunohistochemical analysis of inflamed mucosal biopsies revealed that expression of p38α was abundant in activated macrophages and neutrophils infiltrating bowel mucosa [78]. Using the DSS-induced experimental colitis model in mice, mimicking human inflammatory bowel disease, Hollenbach et al. [79] found that SB203580 improves the clinical score, ameliorates the histological alterations, and reduces mRNA levels of pro-inflammatory cytokines. Interestingly, the NF-κB pathway, activated during colitis induction, was down-regulated by SB 203580 treatment [79].

A human trial using CNI-1493, a synthetic guanylhydrazone, and a potent JNK/p38 MAPK inhibitor, showed a clinical benefit and mucosa healing in patients with severe Crohn’s disease ([80], for a review, see [11,12]).
3.4. Inhibitors of p38 MAPK in brain inflammation and stroke models

Sustained activation of p38α MAPK was observed in activated microglia in the brain after cerebral brain ischemia [81]. SB 203580 significantly reduced infarct size in the gerbil global ischemia model [81]. A second-generation p38 MAPK inhibitor, SB 239063, reduced infarct volume and neurological deficit in rats after transient [82] and permanent [83,84] middle cerebral artery occlusion (MCAO). Piao et al. [85] reported that intraventricular injection of SB 203580 30 minutes before MCAO reduced the infarct volume to 50% of the control and resulted in an improvement of neurological deficits. More interestingly, the infarct volume was significantly reduced when SB 203580 was administered 6 or 12 h after MCAO, which suggests a much wider therapeutic window than with other drugs. The induction of inducible nitric oxide synthase, TNF-α, IL-1β and cyclooxygenase-2 was attenuated by the administration of SB203580 at 6 h after MCAO [85].

A number of pathological, clinical, and therapeutic studies indicate that inflammation is involved in the pathogenesis of Alzheimer’s disease because reactive microglia and pro-inflammatory molecules are present around amyloid β aggregates [14]. Up-regulation of p38 MAPK has been reported in the brains of a transgenic mouse model of Alzheimer’s disease, and Aβ exposure has been shown to stimulate p38 MAPK phosphorylation in microglia in vitro [86,87]. Transgenic mice overexpressing APP751 (a 751-amino acid isoform of β-amyloid precursor protein APP), which have increased inflammatory response, are more vulnerable to ischemic insult [14,88]. When the APP751 mice were treated with SD282, an inhibitor of p38 MAPK, this protected the brain against ischemic injury and abolished the difference in ischemic vulnerability between the APP751-overexpressing and wild type mice. Down-regulation of p38 MAPK activity in the treated mice was associated with decreased expression of iNOS mRNA and AP-1 DNA binding activity, indicating that p38 MAPK is an important upstream regulator of inflammatory pathways that are linked to the increased neuronal vulnerability to ischemic insults [88].

3.5. Inhibitors of the p38 MAPK and JNK signalling pathways as candidate anti-inflammatory drugs

CEP-1347 is a potent inhibitor of mixed lineage kinases (MLKs), a distinct family of mitogen-activated protein kinase kinase kinases (MAPKKK) [89]. It blocks the activation of the c-Jun/JNK apoptotic pathway in neurons exposed to stress and attenuates neurodegeneration in animal models of Parkinson’s disease [90,91]. Recent studies by Lund et al. [92] demonstrated that the MLK inhibitor CEP-1347 reduced cytokine production in primary cultures of human and murine microglia, and in monocyte/macrophage-derived cell lines, stimulated with various endotoxins or the plaque forming peptide Aβ1–40. Moreover, CEP-1347 inhibited brain TNF production induced by intracerebroventricular injection of LPS in mice.

As might be expected, CEP-1347 acted upstream of p38 and c-Jun activation in microglia [92].

Since specific JNK inhibitors became available only recently, the role of JNK in inflammation remains enigmatic, and no specific JNK inhibitor has entered clinical evaluation. SP600125, developed by Celgene, is an inhibitor of JNK2 that does not interfere with the ERK or p38 MAP kinase pathways and reduces paw swelling in a rat model of inflammatory arthritis [93]. CEP-1347 is a potent anti-inflammatory compound in experimental arthritis [94].

Another potential anti-inflammatory drug is CNI-1493, a synthetic guanylylhydrazone which inhibits the phosphorylation of both p38 MAP kinase and JNK. CNI-1493 can suppress macrophage activation and the production of several pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and macrophage inflammatory protein 1α and 1β [95,96]. CNI-1493 has shown protective effects in animal models of endotoxic shock [97] and rheumatoid arthritis [98]. In a trial in Crohn’s disease patients, CNI-1493 administration resulted in a significant decrease in severe Crohn’s disease and increase in mucosal healing [80]. Since CNI-1493 inhibited more potently JNK phosphorylation than p38 in LPS-stimulated peripheral blood mononuclear cells in vitro, and in mucosal inflammatory cells in vivo, the observed beneficial effects may be more related to JNK inhibition.

4. Immunosuppressants as potential anti-inflammatory drugs affecting MAPK signalling pathways

The immunosuppressants: cyclosporin A (CsA), FK506 and rapamycin are short peptides routinely used to block graft rejection in transplantation of organs and bone marrow [99]. They bind to specific intracellular proteins called immunophosphatins: CsA binds to cyclophilin, FK506 and rapamycin bind to FKBP (FK506-binding protein). Both types of complexes bind to the regulatory subunit of calcineurin and thus inhibit its activity. Calcineurin (PP2B, PP3) is a calcium- and calmodulin-dependent threonine/serine phosphatase. Calcineurin is the crucial enzyme engaged in the activation of immune system cells and thus inhibition of its activity is the major mechanism of immunosuppressant action [100].

In addition to their immunosuppressive properties, FK506 (and to a lesser extent CsA) may exert a powerful neuroprotective and neuroregenerative action in animal models of neurologic diseases such as: traumatic brain injury, spinal cord injury, optic nerve crush, antiretroviral toxic neuropathy, rodent models of Parkinson’s disease, and stroke (for reviews, see [101,102]). CsA and FK506 display neuroprotective effects in focal and global ischemia in rats, gerbils, and primates. We found that a single systemic injection of FK506 1 h after MCA occlusion resulted in the decrease of microglia and astrocyte activation, which correlated with a decrease of brain injury and neurologic deficits [103]. Furthermore, FK506 treatment attenuated ischemia-induced increase in IL-1β and TNF-α mRNA expression in rats. In primary astrocyte cultures, FK506 treatment decreased the levels of TNFα and IL-1β mRNAs, while the levels of TGF-β1 and Interleukin-6 mRNAs were...
unaffected ([104], and unpublished). Furthermore, lipopolysaccharide-induced accumulation of IL-1β and TNF-α mRNAs, and IL-1β protein synthesis were inhibited by FK506 in a dose-dependent manner in cultured microglia [103].

Since it has been shown that cytokine expression in LPS-treated microglia is controlled by MAP kinases, we studied FK506 effects on MAPK signalling. Microglial cultures stimulated with LPS showed rapid and transient (1–3 h) activation of three different MAPKs (p38, ERK1/2, JNK), and a later increase of IL-1β, TNF-α, and IL-6 mRNA levels, consistent with a possible relationship between MAPK and pro-inflammatory cytokine expression. We found that FK506 strongly inhibited p38 MAPK and JNK activation induced by LPS in microglial cells. CsA applied at similar doses did not recapitulate the FK506 effects on MAPK signalling and cytokine expression (unpublished). Our findings demonstrate that immunosuppressants may trigger and modulate the signalling pathways critical for inflammation.

In the past, due to its immunosuppressive effects CsA underwent extensive clinical evaluation for the treatment of Crohn’s disease and ulcerative colitis. Initial studies employed high-dose intravenous CsA and have demonstrated success for the treatment of chronic intestinal inflammation [105,106]. However, attempts to convert high-dose CsA to lower doses for long-lasting treatment have not been effective. This may have resulted from unfavourable effects of CsA on non-immune cell populations, in particular the vascular endothelium [107].

Immunosuppressants have not been considered as MAPK signalling inhibitors. However, Matsuda et al. [108] reported that the FK506–FKBP complex suppresses the activation of the JNK and p38 pathways in T lymphocytes by interfering with signal transduction at the level of MAPKKK. Although CsA had a similar inhibitory effect, these effects were apparently independent of calcineurin inhibition. The mechanism of the inhibitory action of FK506 on MAPK signalling as well as its anti-inflammatory potential awaits further studies. However, an advantage of using FK506 as an anti-inflammatory drug is its combined action on JNK and p38 MAPK pathways, the well-known pharmacokinetics, and long-term studies of potential side-effects in humans.

5. Conclusions and perspectives

Understanding of the signal transduction mechanisms and gene regulation involved in inflammation provides numerous opportunities for the discovery of novel compounds which may be useful in therapy of inflammatory disorders. The inhibitors of p38 MAPK and JNK signalling pathways are noticeably attractive because they are capable of reducing both the synthesis of pro-inflammatory cytokines and their intracellular signalling. Moreover, many of these drugs are small molecules that can be administered orally and a growing number of studies demonstrate their therapeutic benefits in animal models of chronic inflammatory disease [109].

Major challenges in potential application of MAPK inhibitors as anti-inflammatory therapeutics are first of all drugs specificity and, secondly, potential side effects during chronic treatment. Novel drugs targeting activity of p38 MAPK or second generation inhibitors have been improved in respect to drug specificity against the p38 MAPK α- and β-isoforms. For example, SB 230963, a second generation p38 MAPK inhibitor, [trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl)-5-(2-methoxypyridimidin-4-yl)imidazole, displays high-affinity binding to p38 MAPK and potent inhibitory activity against the α- and β-isoforms (IC50 = 44 nM), with no activity against the γ- and δ-kinase isoforms (IC50 up to 100 µM) or many closely related kinases, including lipid kinases, tyrosine kinases, Erk, and JNK (IC50 up to 10 µM) [110]. A novel compound, RWJ 676557 [(4-[4-(4-fluorophenyl)-1-(3-phenylpropyl)-5-(4-pyridinyl)-1Himidazol-2-yl]-3-butyn-1-ol)], inhibits the enzymatic activity of the recombinant p38 α- and β-isoforms (IC50 = 30 nM) and the production of TNF-α and IL-1β by LPS-stimulated human monocytes/macrophages, with IC50 values of 3 and 11 nM (for SB 203580, IC50 = 0.6–1.5 µM). It has no significant activity against a number of other enzymes, including tyrosine kinases p56Lck and c-Src, PKA, ERK-2 [76].

There is a considerable concern about potential side effects related to prolonged treatment with MAPK inhibitors as anti-inflammatory drugs. The role of inflammatory responses and cytokines in the innate resistance to bacterial infections is well established. Long-term inhibition of MAPK may impair normal bactericidal mechanisms in neutrophiles and decrease bacterial clearance during infection. MAP kinases play an important role in key cellular processes and are necessary for immune system homeostasis [31]. Further studies of the sequence and mediators of the inflammatory cascade should provide more information regarding conditions under which the damaging role of inflammation overrides the protective role. So far MAPK inhibition-related side effects (mainly hepatotoxicity) and unfavourable events in animal models are limited. But the reported clinical side-effects of MAP kinase inhibitors have not precluded further clinical development.

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