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# World Journal of *Pharmacology*

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## INDEXING/ABSTRACTING

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## FLYLEAF

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*REVIEW*

# Role of mitogen- and stress-activated kinases in inflammatory arthritis

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## Abstract

Lysophosphatidic acid (LPA) is a pleiotropic lipid mediator that promotes motility, survival, and the synthesis of chemokines/cytokines in human fibroblast-like synoviocytes (FLS) from patients with rheumatoid arthritis. LPA activates several proteins within the mitogen activated protein (MAP) kinase signaling network, including extracellular signal-regulated kinases (ERK) 1/2 and p38 MAP kinase (MAPK). Upon docking to mitogen- and stress-activated kinases (MSKs), ERK1/2 and p38 MAPK phosphorylate serine and threonine residues within its C-terminal domain and cause autophosphorylation of MSKs. Activated MSKs can then directly phosphorylate cAMP response element-binding protein (CREB) at Ser133 in FLS. Phosphorylation of CREB by MSKs is essential for the production of pro-inflammatory and anti-inflammatory cytokines. However, other downstream effectors of MSK1/2 such as nuclear factor-kappa B, histone H3, and high mobility group nucleosome binding domain 1 may also regulate gene expression in immune cells involved in disease pathogenesis. MSKs are master regulators of cell function that integrate signals induced by growth factors, pro-inflammatory cytokines, and cellular stresses, as well as those induced by LPA.

Key words: Lysophosphatidic acid; Mitogen activated protein kinase; Chemokines; Cytokines; Mitogen- and stress-activated kinases; Inflammation; cAMP response element-binding protein; Arthritis

**Core tip:** Extracellular signal-regulated kinases 1/2 and p38 mitogen activated protein kinase cascades are activated in response to stimulation with inflammatory stimuli, including lysophosphatidic acid, and are able to activate mitogen- and stress-activated kinase (MSK) 1 and MSK2 in human synovial fibroblasts. MSKs then

phosphorylate the transcription factor cAMP response element-binding protein (CREB), leading to the production of pro-inflammatory and anti-inflammatory cytokines. In addition to CREB, many other downstream effectors of MSK1/2 such as nuclear factor-kappa B, histone H3, the E3 ubiquitin ligase, Tripartite motif containing 7 and high mobility group nucleosome binding domain 1 have been reported and suggested to play important functions in immunity and disease states, including arthritis.

## INTRODUCTION

Mitogen- and stress-activated protein kinases (MSKs) were first identified as efficient cAMP response element-binding protein (CREB) kinases in 1998[1]. For the past 17 years, MSKs have been investigated thoroughly as regulators of gene expression at multiple levels[2]. The known function of MSKs is mainly phosphorylation of transcription factors, chromatin-associated proteins and ubiquitin ligase. MSKs are activated in response to mitogenic signals [*e.g.*, serum, epidermal growth factor and fibroblast growth factor, lysophosphatidic acid (LPA)], neurotransmitters, progesterone, cellular stresses (*e.g.*, UV-irradiation, oxidative stress, arsenite, metals and retinoic acid), and other signals from pro-inflammatory cytokines (*e.g.*, TNF-$\alpha$), as well as PAMP[3]. Through binding to G protein-coupled receptors, cytokine/chemokine and growth factor receptors, or activation of stress sensors, all these stimuli activate various mitogen activated protein kinase (MAPK) signaling pathways (p38 MAPK and ERK). Activation of ERK1/2 and p38 MAPK directly or indirectly through the phosphorylation of MSKs regulates the function of transcription factors and nuclear proteins involved in gene transcription (Figure 1). The substrates of MSKs including, CREB, ATF-1, NF-$\kappa$B p65, Histone H3, and high mobility group nucleosome binding domain 1 (HMGN1), have been extensively studied and validated in cells silenced for the expression of MSK1 and/or MSK2[2]. Another protein, the E3 ubiquitin ligase named Tripartite motif containing 7 was recently reported to be a target of MSK1 using cell silencing approaches, thereby highlighting the crosstalk between different post-translational protein modifications[4]. The roles of MSKs downstream of ERK1/2 and p38 MAPK and their important functions in immunity and disease states, including arthritis, have been highlighted in the book edited by Arthur[3].

Rheumatoid arthritis (RA) is a severe, chronic and systemic inflammatory disease. Infiltration of multiple blood-derived cells (macrophages, dendritic cells, T cells, B cells, neutrophils, platelets[5]) into inflamed joints, high levels of cytokines/chemokines, production of lipid mediators and matrix metalloproteinases (MMPs) in synovial joints, synovial cell proliferation leading to synovium thickening, and pannus formation are the hallmarks of RA[6]. All these features eventually lead to cartilage dysfunction, damage to adjacent tissues, and deformation of joints with associated chronic pain. At the present time, medical therapy for RA uses conventional disease-modifying anti-rheumatic drugs such as corticosteroids and methotrexate, anti-malarials, and TNF inhibitors alone or in combination with methotrexate. New strategies targeting other cytokines, like IL-1[7,8] and IL-6[9,10], or B cell marker CD20[11] are also approved for RA treatment. Inhibitors of specific protein kinase pathways also hold potential in the treatment of chronic autoimmune diseases. More recently the JAK1/3 inhibitor tofacitinib, which suppresses inflammation driven by immune cells through inhibition of JAK/STAT, was approved by the Food and Drug Administration for the treatment of RA[12]. Although anti-cytokine therapies provide significant benefits for RA patients, there is still a substantial subset of nonresponsive patients as well as patients who cannot tolerate the current therapy[13]. Presently, researchers focus more on the cellular pathways of inflammation to search for new therapeutic targets for the treatment of autoimmune diseases such as RA[14]. MAPKs are potential targets to treat RA because of their important role in regulating cell proliferation, apoptosis, cytokine and MMP expression[15]. The functions of MSKs as important nuclear signaling kinases phosphorylated by MAPKs and regulators of inflammatory gene transcription were investigated extensively during the past decade. More extensive reviews are found elsewhere[16,17]. In this article, we will summarize current knowledge on MSK signaling in inflammatory arthritis and describe its potential roles in amplification and perpetuation of inflammation.

## MSK STRUCTURE AND ACTIVATION MODE

The kinase domains composing MSK1/2 include a C-terminal and N-terminal kinase domain, harboring several phosphorylation sites[16,17] (Figure 1). Human MSK1 can be activated by MAPK at Ser360, Thr581 and Thr700 located in the C-terminal domain[18]. Phosphorylation of the C-terminal domain induces a conformational change in MSK1, which permits autophosphorylation on Ser212, Ser376 and Ser381 by the C-terminal kinase domain and phosphorylation of MSK substrates by the N-terminal kinase domain[2,18]. Compared with ribosomal S6 kinases (RSKs), the main difference is that RSK is activated by ERK whereas MSK can be activated by both ERK and p38 MAPK through a closely related mechanism that reflects the common domain structure of MSKs and RSKs[19]. The molecular docking interaction

**Table 1 Specificity and selectivity of inhibitors currently being used to inhibit mitogen- and stress-activated protein kinase 1/2**

| MSK inhibitors | IC$_{50}$ | Mechanism of action | Ref. |
| --- | --- | --- | --- |
| SB747651A | 0.5 nmol/L | Selectively targets MSK1/2 | [45,100,101] |
| | | Inhibits the N-terminal kinase domain of MSKs | |
| | | > 300-fold selectivity over RSK1 and > 3000-fold selectivity over GSK3 | |
| Ro-31–8220 | 8 nmol/L | Inhibitor of PKC, MSK1, RSK, S6K1, GSK3 | [85,102] |
| H89 | 120 nmol/L | Inhibitor of MSK1, S6K1 and ROCK-II, PKA | [85] |

MSK: Mitogen- and stress-activated protein kinase; RSK1: 90 kDa ribosomal S6 kinase (p90rsk), also known as MAPKAP-K1(RPS6KA1); GSK3: Glycogen synthase kinase 3; PKC: Protein kinase C; S6K1: Ribosomal protein S6 kinase beta-1; ROCK-II: Rho-associated protein kinase 2; PKA: Protein kinase A.

between p38 MAPK or ERK1/2 and MSKs has been clearly highlighted previously[19,20] and will not be further discussed in this review.

## MSK IN IMMUNE AND SYNOVIAL CELLS

### T cells
The key evidence for the aberrant pathway of T cell activation in the initiation and perpetuation of RA is the association between disease pathogenesis and HLA-DRB1[21,22]. Th1 cells expressing IFNγ and TNF-α are present in RA synovial tissues[21,23]. Data from animal models of arthritis suggest that IL-17-producing CD4$^+$ T cells (Th17 cells), also contribute to the inflammatory processes[24-26]. The p38α MAPK-MSK1/2 axis was reported to induce IL-17 synthesis by CD4$^+$ T cells in experimental models of autoimmune diseases[27]. The absence of the *msk1/2* gene resulted in the failure to produce IL-17 by murine lymphocytes isolated from lymph node and blood[27]. The potential role of MSK1/2 in the regulation of gene transcription downstream of p38α MAPK signaling in T cells is illustrated by the LAT (linker for activation of T cells) signalosome that propagates signals through branching of several signaling pathways including that of MAPK[28]. In T cells, MSK is the major kinase responsible for CREB phosphorylation in response to TCR activation, and MSK1/2 knockout mice showed reduced T cell proliferation in the presence of IL-2[29]. In this study, the authors pointed out that MSK1/2 are highly expressed in the thymus and the spleen, and that spleens from MSK1/2 knockout mice contain fewer T cells. Data for tissue-specific patterns of mRNA expression available at the Scripps Research Institute BioGPS Website and Database indicate that MSK1 (gene symbol: RPS6KA5) but not MSK2 (gene symbol: RPS6KA4) is highly expressed in CD19$^+$ B cells, CD4$^+$ T cells, CD8$^+$ T cells, and CD56$^+$ NK cells compared to other tissues and cell types. Hence, it is possible that MSK1 and MSK2 have different functions (*i.e.*, substrate specificity) in those cells.

### B cells
CD20$^+$ B cells are enriched in the RA synovium and their functions mainly include autoantibody production, T cell interaction and cytokine production[30]. MSK1/2

deficiency has no significant effect on T cells or B cell development[29]. At present, we do not know what impact MSK1 and/or MSK2 deficiency has on T/B cell interaction, cytokine/chemokine production by B cells, and mature B cell proliferation. Mn$^{2+}$ induced apoptosis of human lymphoma B cells through the activation of caspase-8[31]. This study using specific pharmacological inhibitors and dominant-negative mutants of p38α MAPK and MSK1 showed the p38α MAPK-MSK1 signaling pathway, but not Fas-associated death domain protein, drives B cell apoptosis. Nevertheless, the mechanism of how the p38α MAPK-MSK1 axis regulates B cell apoptosis is not clear given that caspase-8 does not associate with MSK1 and is not a substrate of MSK1[31]. Another study showed that TGFβ mediated apoptosis in human Burkitt lymphoma B cells through caspase-8 activation downstream of p38α MAPK, but the possible contribution of MSKs to this effect is not yet known[32].

### Neutrophils
Neutrophils constitute 90% of the cells in RA synovial fluids[33]. The crucial roles of neutrophils in inflammation, inflammatory diseases, and systemic autoimmune diseases have been thoroughly reviewed[34-38]. The main functions of neutrophils include phagocytosis, degranulation, production of antimicrobial peptides and proteins, production of reactive oxygen species, and NETosis (release of neutrophil extracellular traps)[35,39]. Khandpur *et al*[40] showed enhanced NETosis of circulating and synovial fluid neutrophils from RA patients, compared to those from osteoarthritis patients or healthy individuals. In neutrophils, a role for p38 MAPK has also been reported in chemotaxis[41], regulation of apoptosis[42], as well as cytokine/chemokine and MMP production[43,44]. The p38 MAPK-MSK1 axis contributes to chemokine production through CREB activation in LPS-stimulated human neutrophils[44]. In this study CREB was presumably phosphorylated by MSK1, but the data require further validation since the authors used Ro-31-8220, a non-selective inhibitor of MSK1[45] (Table 1). There is another report showing that neutrophil stimulation with sphingosine-1-phosphate (S1P) induces p38 MAPK and ERK-dependent phosphorylation of MSK1 to control the secretion of IL-8[46].

Activation of the ERK and p38 MAPK pathways has
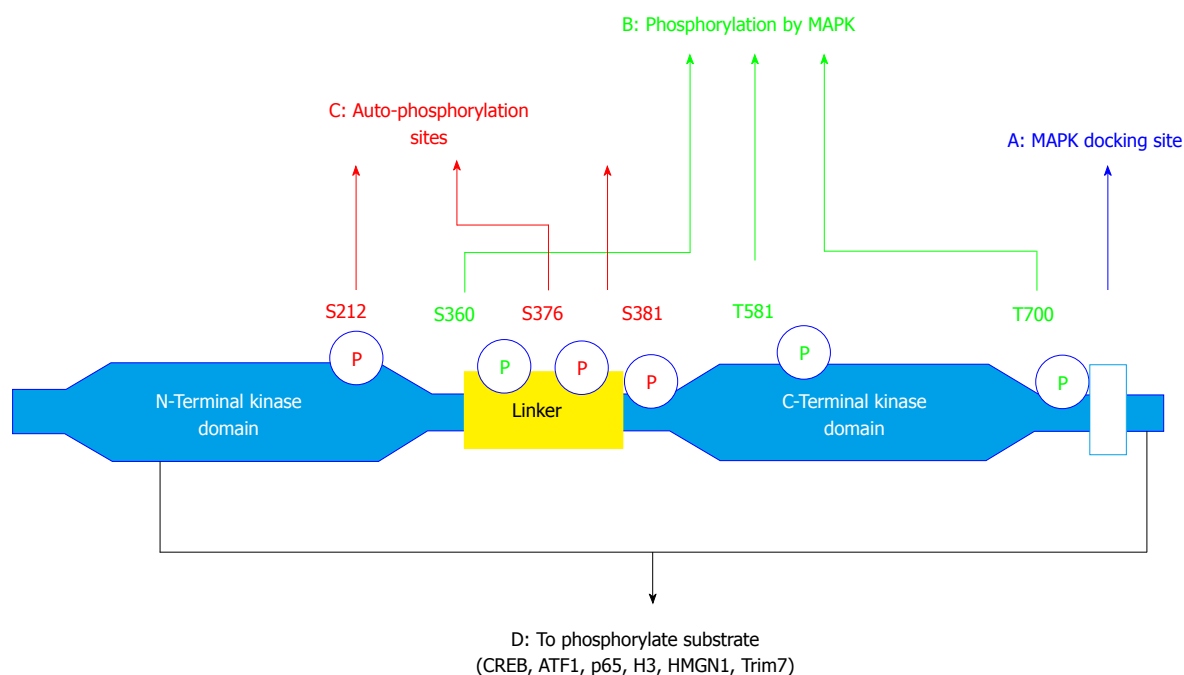
**Figure 1  Schematic representation of human mitogen- and stress-activated protein kinase 1 activation.** A: MAPKs bind to the MAPK-docking site in the MSK C-terminal domain (blue); B: Human MSK1 is activated by MAPK-dependent phosphorylation at Ser360, Thr581 and Thr700 located in the C-terminal domain (green); C: Phosphorylation of the C-terminal domain induces a conformational change in MSK1, which permits autophosphorylation at Ser212, Ser376 and Ser381 by the C-terminal kinase domain (red); D: Phosphorylation of MSK substrate (validated substrates include CREB, ATF-1, p65, H3, HMGN1 and Trim7) by the N-terminal kinase domain (black). MSK1: Mitogen- and stress-activated protein kinase 1; MAPK: Mitogen-activated protein kinases; CREB: cAMP response element-binding protein; ATF-1: Activating transcription factor 1; HMGN1: High mobility group nucleosome binding domain 1.

been reported in human neutrophils stimulated with chemoattractants, pro-inflammatory cytokines, and Fcγ receptor ligands[47], and their activation is also required for the respiratory burst in TNF-α and GM-CSF primed cells[48]. As MSK1 is phosphorylated by ERK1/2 and p38 MAPK in neutrophils under certain conditions, we cannot deny a role for MSKs in the signaling pathway leading to a coordinated pattern of cytokine/chemokine gene expression induced by various stimuli.

*Fibroblast-like synoviocytes*
Fibroblast-like synoviocytes (FLS) play a substantial role in many pathologic events in inflammatory arthritis. As a key component of the hyperplastic rheumatoid pannus, combined with their invasive phenotype, FLS have a major role in the initiation and perpetuation of destructive joint inflammation[49]. As passive responders, FLS in RA secrete cytokines/chemokines, lipid mediators of inflammation, a subset of extracellular matrix remodeling enzymes, and express adhesion molecules. Somatic mutations and epigenetic alterations associated with signaling anomalies may also contribute to invasive behavior, resistance to apoptosis, and production of inflammatory cytokines (reviewed in[49]). We demonstrated that Ser376 on MSK1 and possibly Ser360 on MSK2 were transiently phosphorylated in RAFLS shortly after stimulation with LPA[50] and TNF-α as well (unpublished data). A specific inhibitor of MSKs (SB747651A) or silencing of MSK1 and/or MSK2 with siRNAs significantly reduced LPA-induced

chemokine secretion (IL-8 and MCP-1) and CREB phosphorylation at Ser133[50]. FLS priming with TNF-α for 8 h prior to LPA stimulation consistently increases the phosphorylation of MSK1/2 at Ser376/Ser360 (unpublished data), as well as IL-8, IL-6, and MCP-1 secretion[50,51]. These data suggest an important role for MSKs in LPA signaling which leads to inflammatory cytokine/chemokine secretion by FLS in RA. A possible explanation for transient MSK1/2 phosphorylation could be the activity of a protein phosphatase such as protein phosphatase 2Cδ, which has been reported to be phosphorylated by ERK and to associate with MSKs[52], or dephosphorylation of ERK1/2 and p38α MAPK by dual-specific phosphatase 1 (DUSP1)[53]. Further work is needed to pinpoint the phosphatases that regulate the p38α MAPK-MSK1/2 signaling axis in FLS.

To date little is known about the mechanism of how TNF-α drastically enhances the secretion of chemokines in response to bioactive lipids such as LPA and S1P. Early studies showed correlation between chemokine synthesis and increased expression of a subset of LPA and S1P receptors by cultured FLS or the lining tissue of mouse air pouch when stimulated with TNF-α[54]. Both S1P and LPA promote chemokine secretion, and p38 MAPK, ERK1/2 and Rho kinase activation in FLS[55]. Hence, increased expression of certain LPA receptors (LPA1 and LPA3) and of S1P receptors by cells exposed to an inflammatory environment may contribute, at least in part, to enhanced intracellular signals that lead to further activation of MSK and CREB.

### Macrophages

By producing various pro-inflammatory cytokines/chemokines macrophages play a critical role in cartilage and bone destruction in inflammatory arthritis[56,57]. There is an imbalance between inflammatory and anti-inflammatory macrophages in the RA synovium[58], and more information on how macrophages contribute to RA disease activity at both the local and the systemic levels can be obtained by reference to other reviews[59-61]. LPS-mediated activation of MSK1 and MSK2 was associated with COX-2 expression and IL-1β secretion in macrophages[62]. MSK activation is not restricted to TLR4 signaling, as Pam3CSK4 (TLR1/2 agonist), lipoteichoic acid (TLR2 agonist), CpG-DNA (TLR9 agonist) and dectin-1 agonist stimulation all phosphorylated MSK1 at Thr581[63]. Inhibition of early expression of COX2 in MSK1/2 knockout macrophages was confirmed by another study, but induction of COX-2 protein and prostaglandin secretion was detected at later time points due to reduced LPS-mediated production of IL-10 and increased COX-2 mRNA stability in the absence of IL-10[64]. By regulating the CREB/ATF-1 dependent transcription of DUSP1 and of IL-10, MSK1 and MSK2 are also part of a negative feedback loop that limits TLR4-driven inflammation. Hence, the absence of this feedback loop may explain why LPS-mediated expression of TNF-α, IL-6, IL-12, and late expression of COX-2 are increased in MSK1/2 deficient macrophages[63]. In line with those studies, it was reported that stimulation of MSK1/2 knockout macrophages with zymozan particles reduced the secretion of IL-10 and increased that of IL-12[65]. So far there is no information on the role of MSK1/2 in mouse models of arthritis but it would be interesting to evaluate the impact of MSK knockdown on disease onset, severity and duration as TNF-α, IL-1β, IL-6 and chemokines are produced by various cell types including macrophages and other immune cells[66]. MSKs may limit inflammation since mice that lack MSK1 and MSK2 produce less IL-10 and IL-1 receptor antagonist, which provide crucial negative-feedback loops in response to LPS[64,67]. A study investigating genetic variations in the p38 MAPK signaling network in RA patients identified SNPs in MSK1 and MSK2 that were associated with anti-TNF treatment response[68]. Further analysis suggests that the MSK2 genetic variant has a recessive effect whereas other SNPs in proteins of the p38 MAPK signaling pathway have a dominant effect on the change in DAS28[68]. However, the impact of these SNPs on MSK protein expression and activation has not been investigated.

## ROLES OF MSK IN CYTOKINE/CHEMOKINE PRODUCTION

MSKs play a versatile role through the phosphorylation of transcription factors and nuclear proteins that up-regulate the expression of pro-inflammatory and anti-inflammatory genes including chemokines/cytokines and signaling proteins[16,17]. *TNF-α*, *IL-6*, *IL-2*, and *IL-10* genes share in common a CRE element in the core promoter region that is required for CREB binding and gene transcription[69,70]. However, phosphorylation of CREB on Ser133 by MSKs has a greater effect on the induction of CREB-dependent immediate-early genes than that induced by protein kinase A (PKA), possibly due to differential recruitment of CREB co-activator proteins[71]. Activation of ERK1/2 also leads to histone phosphorylation and Sp1 transcription factor binding to the IL-10 promoter[72]. As validated substrates of MSKs, histone H3 and HMGN1 may also contribute to immediate-early gene expression through various mechanisms[2].

The expression of IL-8 (CXCL8) is controlled by three different mechanisms: derepression of the gene promoter; transcriptional activation of the gene by NF-κB and JUN-N-terminal protein kinase pathways; and stabilization of the mRNA by the p38 MAPK pathway[73]. In FLS from RA patients the production of IL-8 is upregulated by approximately 100-fold in response to TNF-α or IL-1β[5]. Inhibitors of p38 MAPK inhibit the functional responses to these cytokines including the production of IL-8[74]. This study suggests positive feedback loop mechanisms that lead to activation of the p38 MAPK pathway and long term IL-8 secretion, which recruit neutrophils to the inflammatory sites. We demonstrated that LPA-induced production of IL-8 is inhibited by inhibitors of p38 MAPK and MSK, as well as silencing of MSK1/2 and CREB in FLS[50,51]. The mechanism by which MSK regulates the transcription of CREB-dependent genes such as *IL-8* and *MCP-1* as well is not clear[71]. In RAFLS, NF-κB p65 subunit, but not C/EBP-β or AP-1, dominantly regulates *IL-8* gene expression under IL-1β stimulation[75]. Whether phosphorylated CREB needs to recruit co-activation proteins (CBP/p300)[76] or synergizes with other transcription factors such as NF-κB p65 subunit[75,77], C/EBP-β[78,79], or AP-1[80-82] to regulate IL-8 expression in FLS requires further investigation.

## THE INHIBITORS OF MSK

Two MSK inhibitors have been used to study various MSK functions, including the secretion of cytokines[1,83-90]. However, these inhibitors are not selective for MSKs and inhibit many other kinases, including PKA[45]. This is a major limitation since these compounds show better selectivity for PKA which targets CREB, ATF1, RARα and nuclear factor-kappa B (NF-κB) p65 subunit shown to be phosphorylated by MSKs[2,17,91]. The selectivity of new MSK inhibitor SB-747651A was evaluated *in vitro* and shown to have superior selectivity for MSKs than that of H89 and Ro31-8220[45]. In this study, the authors showed that Ro31-8220 reduced LPS-induced TNF-α secretion that cannot be attributed to MSK inhibition. In contrast, inhibition of MSK in LPS-stimulated macrophages reduced IL-10 secretion and enhanced that

of IL-12 as previously reported in MSK1/2 knockout cells[63,64]. In oral squamous cell carcinoma, SB-747651A was found to inhibit the phosphorylation of NF-κB p65 subunit[92]. We demonstrated that SB747651A inhibits LPA-mediated chemokine synthesis through inhibition of CREB phosphorylation[50]. SB-747651A inhibits CREB phosphorylation without affecting MSK1 phosphorylation at Thr581 (a critical site for MSK1 activation)[64]. Autophosphorylation of Ser212 and Ser376 in MSK1 is poorly affected by SB-747651 thereby suggesting that the inhibitor targets the N-terminal kinase domain of MSKs[45]. Although SB747651A showed improved selectivity, off target effects cannot be totally excluded since other kinases like RSK1, p70RSK and Rho-associated protein kinase 2 (ROCK-Ⅱ) are inhibited by the compound[45].

Inhibition of p38α MAPK showed efficacy in animal models of arthritis but failed in clinical trials[93-96]. Inhibition of p38 MAPK initiates an imbalance between anti-inflammatory and pro-inflammatory processes which excludes this kinase from drug targeting in autoimmune diseases[97]. This is illustrated by the fact that MSKs, the downstream targets of p38 MAPK, differentially regulate the synthesis of two important anti-inflammatory cytokines, IL-10 and IL-12, as a negative feedback loop in inflammation[63]. From the experience of cancer therapy, monotherapy using signaling inhibitors such as MEK-ERK is not permanently effective, as cells may become resistant to the inhibitor by different mechanism[98]. As the aggressive characteristics of RA synoviocytes were viewed as reminiscent of neoplastic tissue[99], and MAPK signaling plays an important role in cell proliferation and cytokine production, it will be necessary to study well the signaling pathways downstream of MSKs to better understand their dual roles in inflammation.

## CONCLUSION

Several inflammatory cytokines and lipid mediators of inflammation activate MSK1/2 signaling downstream of p38 MAPK and ERK1/2. Inhibitors of MSK1/2 have effects on different cell types involved in the pathogenesis of RA, which could provide an important advantage in treatment. Inhibitors of MSK inhibit the production of cytokines/chemokines as well as the responses induced by these pro-inflammatory mediators thereby limiting the activation and/or the recruitment of immune cells to sites of inflammation. However, as reported for other inhibitors of the MAPK pathway, targeting MSK in arthritis may have undesirable effects due to inhibition of other kinase pathways or regulation of complex positive and negative feedback loops that could induce imbalance in the production of pro-inflammatory and anti-inflammatory mediators. Screening for more selective inhibitors or developing isoform-specific inhibitors of MSK1 and MSK2 is required to establish applicability as a drug in the future. Furthermore, more research will be necessary to identify targets downstream of MSK1/2. Understanding how CREB interplays with other transcription factors such as NF-κB or other MSK-dependent pathways regulating protein stability through ubiquitinylation may enable the development of drugs that have less adverse effects for treatment of chronic inflammatory diseases.

## ACKNOWLEDGMENTS

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*MINIREVIEWS*

# Quinolone-based first, second and third-line therapies for *Helicobacter pylori*

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## Abstract

*Helicobacter pylori* (*H. pylori*) is a very common bacterium that infects about 50% of the world population in urban areas and over 90% of people living in rural and developing countries. Fluoroquinolones, a class of antimicrobials, have been extensively used in eradication regimens for *H. pylori*. Levofloxacin is the most commonly used, and in second-line regimens, is one of the most effective options. However, an increasing resistance rate of *H. pylori* to fluoroquinolones is being observed, that will likely affect their effectiveness in the near future. Other novel fluoroquinolone molecules, such as moxifloxacin, sitafloxacin, gatifloxacin and gemifloxacin, have been proposed and showed encouraging results *in vitro*, although data on their clinical use are still limited. Further studies in large sample trials are needed to confirm their safety and efficacy profile in clinical practice.

**Key words:** *Helicobacter pylori*; Eradication regimens; Fluoroquinolones; Antibiotic resistance; Levofloxacin; Rescue treatments

**Core tip:** In the present minireview, we analyzed current evidence about the use of fluoroquinolones in first, second and third-line eradication regimens for *Helicobacter pylori* (*H. pylori*). The increasing resistances to levofloxacin are a worrying issue, that confirm the need to use this drug with proper care. We analyzed the current use of fluoroquinolones in first-line and rescue regimens, underlining possible pitfalls and mistakes that could be avoided in clinical practice. Novel molecules have been investigated, that could offer an interesting tool to combat *H. pylori*.

# INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a very common bacterium that infects about 50% of the world population in urban areas and over 90% of people living in rural and developing countries[1,2]. The imperative to treat *H. pylori* infection lies in the fact that it is a known risk factor for benign[3] (chronic gastritis and peptic ulcer disease) and malignant [adenocarcinoma and mucosa-associated lymphoid tissue lymphoma] gastric disorders[4]. Indeed, bacterial eradication may change the natural course of these diseases and prevent their malignant evolution[5].

Currently, first-line therapies (triple, sequential or concomitant regimens) are able to achieve eradication in about 80% of cases but when these fail, a second-line regimen is necessary. In this case, Maastricht Ⅳ guidelines advise a levofloxacin-containing triple therapy or a bismuth-based quadruple regimen[6]. Since bismuth is not available worldwide and in some countries an excessive number of tablets of tetracycline formulations may be needed to obtain a therapeutic effect[7], fluoroquinolone-containing triple therapies are being adopted with increasing frequency several fluoroquinolone-based protocols, mainly including levofloxacin, have been proposed and tested in different combinations as first, second and third-line treatment for *H. pylori* eradication[8].

Aim of this review is to depict an all-encompassing scenario of the use of fluoroquinolones for *H. pylori* eradication.

# FLUOROQUINOLONES: MAIN CHARACTERISTICS IN THE *H. PYLORI* CONTEXT

The fluoroquinolone drug class is active against both gram-positive and gram-negative bacteria. It acts by inhibiting DNA gyrase, a type Ⅱ topoisomerase, as well as topoisomerase Ⅳ, an enzyme necessary to separate replicated bacterial DNA, thus inducing a block of cell division[9]. This mechanism explains its effectiveness against *H. pylori*. However, the resistance of this bacterium to fluoroquinolones is due to point mutations in the *gyrA* region[10].

The antibiotics belonging to this class that have been applied for *H. pylori* eradication are moxifloxacin, sitafloxacin and levofloxacin, which is the most commonly employed. Although guidelines recommend its use in second-line regimens, several studies have used this antibiotic in first-line treatment. This last application may theorically not be correctly indicated because of the risk of further increasing antibiotic resistances[11] due to plasmid-mediated horizontally transferable genes[12]. An example of this unfavorable trend has been observed in Asian countries, where the resistance rates are largely above 10%: 18.4% in Vietnam[13], 20.6% in China[14] and 63.3% in Pakistan[15]. Only Malaysia registered 0%[16] and Japan 8.2%[17].

In Europe, the global resistance to levofloxacin, according to a recent multicentric epidemiologic study, is 14.1%[12], with values ranging from 11.7% in Ireland[18] to 29.1% in Germany[19]. This last percentage should be paid particular attention, if we consider that in 2003 a resistance rate of only 3.3% was detected in France[20]. In Italy a single study found resistance in 10.6% of the strains[21]; this result was confirmed by a recent overview that observed a rate of 11.8% in already treated patients[22].

# THE USE OF LEVOFLOXACIN IN *H. PYLORI* ERADICATION REGIMENS

### Second line treatments

Levofloxacin has been mainly employed after failure of a first-line regimen, usually in combination with amoxicillin [levofloxacin-based triple therapy (LTT)] for a variable period lasting from 7 to 10 d[23]. Current literature demonstrates that LTT is still more effective than bismuth-containing quadruple therapy. In a meta-analysis by Di Caro *et al*[24] the overall eradication rates were 76.5% in the LTT group and 67.4% in the quadruple regimen group. The superiority of LTT was more evident when it was administered for 10 d (88.7%) as compared to 7 d (70.6%). In a similar meta-analysis by Gisbert *et al*[25], a better tolerability profile of LTT in second-line treatment as compared to the quadruple regimen protocol including bismuth was reported: adverse events occurred in 0.8% and 8.4% of the two groups, respectively.

Other levofloxacin-based alternative regimens have been proposed as second-line therapies after the failure of clarithromycin-based first-line regimens. The "Sequential with levofloxacin" schedule (SQL) has been used in some trials[26]; amoxicillin is given for the first 5 d and levofloxacin plus metronidazole for the remaining 5 d, but the reported success rate is variable, ranging from 65.4% to 82.5%[27,28]. Prolonging the administration of LTT up to 14 d has been proposed as a strategy to improve the effectiveness. Indeed, in a study from Taiwan, 14-d LTT had a success rate of 90.5%, much better than the 10-d LTT (73.6%)[29].

### First line treatments

The application of levofloxacin in first-line regimens shows a satisfactory outcome. To date, eleven studies[30-40], reported in Table 1, have investigated LTT as first-line treatment. The pooled success rate was 79.1%, with a 95%CI of 77.7%-80.5%. Studies comparing two different proton pump inhibitors (PPI) in this setting showed an equivalent power[32].

Some studies, therefore, had a disappointing eradication rate for LTT, close to 70%. For these reasons, other levofloxacin-based first-line regimens have been proposed: sequential (SQL) and concomitant (CL). SQL was able to eradicate the bacterium in 96% of cases in an Italian study[41], and a similar rate (95.6%)

| Ref. | Year | Country | PPI | Eradicated/enrolled patients | Duration (d) | ITT rate |
|------|------|---------|-----|------------------------------|--------------|----------|
| Qian *et al*[30] | 2012 | China | Esomeprazole | 269/345 | 7 | 78.1% |
| Cuadrado-Lavín *et al*[31] | 2012 | Spain | Omeprazole | 207/250 | 10 | 82.8% |
| Pan *et al*[32] | 2010 | China | Esomeprazole/rabeprazole | 173/199 | 7 | 87.1% |
| Assem *et al*[33] | 2010 | Egypt | Esomeprazole | 381/450 | 7 | 84.7% |
| Erçin *et al*[34] | 2010 | Turkey | Lansoprazole | 66/91 | 14 | 72.0% |
| Liou *et al*[35] | 2010 | Taiwan | Lansoprazole | 320/432 | 7 | 74.0% |
| Chen *et al*[36] | 2010 | China | Esomeprazole | 222/300 | 7 | 74.0% |
| Molina-Infante *et al*[37] | 2010 | Spain | Omeprazole | 380/460 | 10 | 82.6% |
| Castro-Fernández *et al*[38] | 2009 | Spain | All PPI | 97/135 | 10 | 71.8% |
| Rispo *et al*[39] | 2007 | Italy | Esomeprazole | 118/130 | 7 | 90.8% |
| Lee *et al*[40] | 2006 | South Korea | All PPI | 186/267 | 7 | 69.8% |
| Total | | | | 2419/3059 | | 79.1% |

**Table 1  Studies investigating first-line levofloxacin-based triple therapies**

The dose of levofloxacin was 500 mg b.i.d and amoxicillin 1 g b.i.d for all studies. PPI: Proton pump inhibitor; ITT: Intention to treat.

was found in a Turkish study[42]. The lowest rate was recorded in a Spanish trial: 82.6%[37]. Only one Italian study tested a 5-d concomitant therapy[43], including levofloxacin 500 mg b.i.d, tinidazole 500 mg b.i.d and amoxicillin 1 g b.i.d. This trial found an eradication rate of 92.2% at intention-to-treat (ITT) and 96.5% at per-protocol (PP) analysis, similar to the control group, that received classical 10-d sequential therapy, which eradicated the bacterium in 93.3% and 95.5% of cases at ITT and PP, respectively. This study prompts two considerations. The first, that conventional first-line therapies are as effective as levofloxacin-containing regimens. The second, that the large difference between ITT and PP in the concomitant regimen is a consequence of a large number of drop-outs, suggesting that this therapy may be less well tolerated than the classical sequential therapy, most probably due to the major antibiotics "charge" ("load"?). Finally, the data in Table 1 do not demonstrate a better outcome of first-line levofloxacin triple therapy than sequential or concomitant treatments. Based on these considerations, the use of levofloxacin in first-line regimens may not be advantageous, taking into account its limited benefits. Moreover, in case of failure, the clinician may encounter some problems in the choice of a rescue therapy, since this good therapeutic option has already been used[44]. Further support of the recommendation to avoid using levofloxacin in first-line regimens may be provided by the evident, rapid increase of worldwide resistances, as reported above.

### Third line regimens

Current guidelines propose a culture-based approach when several attempts to eradicate *H. pylori* fail[6]. Unluckily, antimicrobial susceptibility testing is not widely and promptly available. Because of this limitation, several studies have assessed the effectiveness of empirical third-line protocols including levofloxacin. Gisbert *et al*[45] reported, in a prospective multicentre study, that in third-line therapy, 10-d LTT achieved eradication in 66% of cases in 2006. In Italy, third-line LTT was effective

in 83% and 75%, in two studies performed about 10 years ago[46,47]. Presumably, these satisfactory results cannot be replicated nowadays due to the increased rate of quinolone resistances. Moreover, when compared to a rifabutin-based triple therapy, LTT was shown to be less effective (71.4% *vs* 57.1%, respectively)[48] even if the combination with tetracycline can improve the LTT success rate. A "quadruple" regimen including bismuth, levofloxacin and tetracycline was effective in 78.9% in a study from Taiwan[49]. This peculiar combination has been tested as second-line therapy in two other trials with an excellent outcome in Turkey and Taiwan (90.6% and 95.8%, respectively)[50,51]. However, the use of a quadruple regimen in second-line therapy strongly restricts its therapeutic potential for third-line use.

## NOVEL MOLECULES: MOXIFLOXACIN, SITAFLOXACIN AND GEMIFLOXACIN

Moxifloxacin is a fourth generation fluoroquinolone. Currently, it is indicated for respiratory infections. However, it has been proposed to treat *H. pylori* infection in some Asian and European trials. The first study was conducted in Germany in 2011, and achieved a 95% success rate in second-line therapy, consisting of a 14-d triple (moxifloxacin plus amoxicillin) regimen[52]. More recently, a multicenter European trial carried out in 250 subjects demonstrated that a second-line 14-d moxifloxacin-based triple regimen was effective in 82.4% of cases[53]. In South Korea, where the use of this drug is extensive, the same treatment was able to cure the infection only in 68.4%[54]. This result strongly supports the hypothesis that a wide consumption of fluoroquinolones may lead to a huge prevalence of resistances. Indeed, a subsequent study[55] from the same area confirmed a similar eradication rate (62.4%). A single Turkish study[56] investigated a quadruple regimen (moxifloxacin, bismuth and tetracycline) in second-line and demonstrated satisfactory results, with a success rate of 82.1%.

Sitafloxacin is another fourth generation fluoro-

quinolone which is currently marketed only in Japan and few other Far East Asian countries. For this reason, only five studies have been performed to investigate its application for *H. pylori* treatment[57-60]. These trials showed that a triple therapy with amoxicillin and sitafloxacin in third-line obtained a success rate ranging from 70% to 90.9%. Nevertheless, further studies are required to assess the effectiveness of this drug[17].

Gemifloxacin is a new quinolone which may be a promising alternative to overcome the problem of *H. pylori* levofloxacin resistances. A recent study from Taiwan showed that gemifloxacin demonstrates inhibitory concentration values only slightly lower than levofloxacin for its antimicrobial activity against *H. pylori* isolates, as well as sensitivity in levofloxacin-resistant strains[61]. Therefore, gemifloxacin may be a powerful quinolone to combat *H. pylori* and offers a future alternative for resistant strains. However, current studies have investigated the effectiveness of gemifloxacin only *in vitro*[62-64], and clinical trials are needed to translate these results to humans. Finally, gatifloxacin has been employed only in few pilot studies as a third-line regimen, showing a success rate ranging from 75% to 84.4%[65,66], but with discordant results when used in first-line[67,68].

## CONCLUSION

Fluoroquinolones-based protocols offer encouraging strategies for the eradication of *H. pylori* infection. According to the current evidence and the Maastricht Ⅳ consensus, they should be used as first-line or "rescue" treatment, depending on geographic areas, since *H. pylori* resistance to these antibiotics is increasing. However, the use of levofloxacin in a first-line regimen has limited benefits and it restricts the therapeutic options in case of failure. Therefore, this antibiotic should generally be confined to second-line treatment. In third-line its use is mainly empirical. Despite the relatively few studies, new fluoroquinolones could offer promising alternative options for resistant strains. Moxifloxacin, sitafloxacin, gatifloxacin and gemifloxacin have been less investigated but may be the most encouraging molecules.

The extension of therapy duration to 14 d improves the eradication rates, but this strategy could have an impact on some public health issues such as tuberculosis management. Indeed, a long exposure to fluoroquinolones may delay the diagnosis of the infection, as well as raising drug resistances[69].

In summary, *H. pylori* eradication guidelines recommend the prescription of levofloxacin as first-line therapy only in areas with high clarithromycin resistances. In second-line regimens, levofloxacin-based protocols are a promising strategy as an alternative to quadruple therapy, when the PPI-clarithromycin-amoxicillin association fails. These protocols, moreover, offer the advantages of efficacy, simplicity, and safety. Finally, as regards third-line and "rescue" protocols, the antibiotic

choice should be guided by antimicrobial susceptibility testing. This can be achieved by culture-based (E-test) and/or molecular-based methods (real-time PCR to detect the presence of a *gyrA* mutation).

On the bases of what has been reported above, fluoroquinolones appear to be a very useful and effective treatment option for *H. pylori* eradication. However, they need to be adopted with some caution, and the criteria for their proper use defined, so as to avoid the risk that abuse could lead to a rapid ineffectiveness.

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