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INVITED ARTICLE

THE DEVELOPMENT OF MK2 INHIBITOR: WHERE DOES IT STAND?

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Abstract

Drug development targeting protein kinases is the second most important group of drug target after G-protein coupled receptor which codes 22% of the druggable human genome. The protein kinases are key players in signal transduction, which regulate many different cellular processes in a tightly controlled manner through reversible phosphorylation. Several drugs that inhibit protein kinases have been in clinical use for the treatment of cancer. Mitogen-activated protein kinase-activated protein kinase 2 (MK2 or MAPKAP KINASE 2) is one such kinase activated by $p38^{MAPK}$, which plays a pivotal role in the regulation of inflammation and associated diseases diversifying it from other $p38^{MAPK}$ regulated signaling pathway. Considering the toxicity and side effects of $p38^{MAPK}$ inhibition, in the last decade, efforts were undertaken to develop different classes of MK2 inhibitor for therapeutic interventions as an alternative to the direct inhibition of $p38^{MAPK}$. This review article describes the biology and mechanism of action of MK2, its role in inflammation and the development of small molecular inhibitor of MK2 highlighting opportunities and challenges in drug development targeting such type of kinases. The development of small molecule MK2 inhibitor will provide a better and safe therapeutic option in future.

Keywords: *MK2, inflammation, cytokines, inhibitor*

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Introduction:

Today's quests for the development of novel drug molecules, pharmaceutical industries are exploring protein kinases as a potential drug target. Along with tough challenge for the selectivity of a drug molecule, protein kinase family offers a huge opportunity for drug discovery. 10–15% of all human genes (~ 3,000) are thought to be druggable based on sequence similarity to those that have already been targeted, of these only ~2% have been successfully targeted with small molecule drugs [1]. About 22% of the druggable human genome codes for protein kinases which is second most important group of drug target [2]. The protein kinases regulate many different cell processes starting from development to growth to aging like cell differentiation, cell cycle and proliferation, migration, survival and senescence etc. All these processes are tightly controlled and one of the main strategies in such control is the reversible phosphorylation of the substrate by protein kinase [3]. A pathological condition arises when controlled signal transduction gets aberrant as a result of a change in one or more protein kinase activity. Among the diseases and disorders associated with abnormal signal transduction are cancer, cardiovascular disease and heart failure, autoimmune diseases, inflammatory condition related diseases, neurological disorders and hormone-related diseases and many more. A number of drugs that inhibits protein kinases have been in clinical use for the treatment of cancer [4]. Imatinib mesylate (Gleevec; Novartis) is one of the examples of such kinase inhibitor for chronic myeloid leukemia and stromal tumor [2].

This review is focused on one such protein kinase - Mitogen-activated protein kinase-activated protein kinase 2 (MK2 or MAPKAP KINASE 2), which is mainly implicated in the inflammatory process diversifying it from other $p38^{MAPK}$ regulated signaling pathway. Literature was searched and retrieved from the PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and ScienceDirect (http://www.sciencedirect.com/), patents describing MK2 inhibitor are not included here as those are discussed elsewhere [5].

MK2: An introduction

MK2 is a protein kinase of serine/threonin class which belongs to MAPKAPK family and a major downstream target of a MAP kinase $p38^{MAPK}$ (p38 α/β) [6, 7]. In 1993 *Stokoe et al*, first cloned a partial human MK2 cDNA and reported a primary structure having a prolin rich region with two putative SH3-binding sites, a catalytic domain, a threonine residue which is phosphorylated by upstream kinase and a nuclear localization signal [8]. Furthermore, it has a nuclear export signal and auto-inhibitory domain as well as other phosphorylation sites which are discussed by *Gaestel* along with structural details and isoforms [9]. Apart from MK2, MAPKAPK family members include MK3 and MK5 [10].

At the resting state MK2 exist as complex with $p38^{MAPK}$ in the nucleus of the cell indicating functional nuclear localization

signal [11], also auto-inhibitory domain tightly binds to catalytic domain making its inactive conformation [12, 13]. Catalytically inactive MK2 stabilizes $p38^{MAPK}$ [14]. Upon phosphorylation by upstream kinase (e.g. MAPK kinase-6) $p38^{MAPK}$ phosphorylates MK2 at the regulatory site T334 with subsequent unmasking of a nuclear export signal leading to nucleocytoplasmic transport of $p38^{MAPK}$-MK2 complex [15-17]. The Phospho-$p38^{MAPK}$ and Phospho-MK2 are mainly localized in cytoplasm. It has been reported that activation of MK2 requires the phosphorylation of any two of the three residues T222, S272, and T334, while the maximum activation is achieved when all three residues are phosphorylated [18]. The basal activity of MK2 in nucleus owing to its auto-phosphorylation [18] may represent a mean for having cytoplasmic substrate specificity only when stimulated. Earlier reported optimal substrate-recognition consensus sequence for MK2 [19] was more refined using a peptide library [20] and finally combining these two results, optimal substrate-recognition motif for MK2 (phosphorylation site on a particular substrate by MK2) was concluded as (L/F/I)-X-R-(Q,S,T)-L-pS/pT-hydrophobic where X denotes all amino acids except S, T, Y or C [9].

Following various stress signaling MK2 is phosphorylated and activated by $p38^{MAPK}$, which further leads to phosphorylation of its substrates like small heat shock protein [21], LIM-kinase [22], CDC25b, CDC25C [20], tristetraprolin [23, 24], 14-3-3zeta [25] and others [9]. MK2 has diversified functions in various cellular processes such as inflammation [26, 27], cytoskeleton reorganization [28-30], cell proliferation [20, 31] and migration [14, 22, 32, 33], transcriptional [34-38] and post-transcriptional regulations [24, 39-41].

Molecular mechanism of MK2 in inflammation

The molecular mechanism regulating inflammation via MK2 involves different ways (Figure 1). The production of master inflammatory cytokine TNF is regulated at the post transcriptional level through mRNA stabilization [42]. RNA binding protein tristetraprolin (TTP) along with its interaction partners regulates the stability/translation of mRNA containing adenylate-uridylate-rich element (ARE) in its 3' -untranslated region (3' UTR) by binding to ARE [43]. TNF mRNA is regulated by MK2 dependent manner [41, 44]. In turn TTP itself is transcriptionally regulated by MK2 via phosphorylation of serum response factor [45].

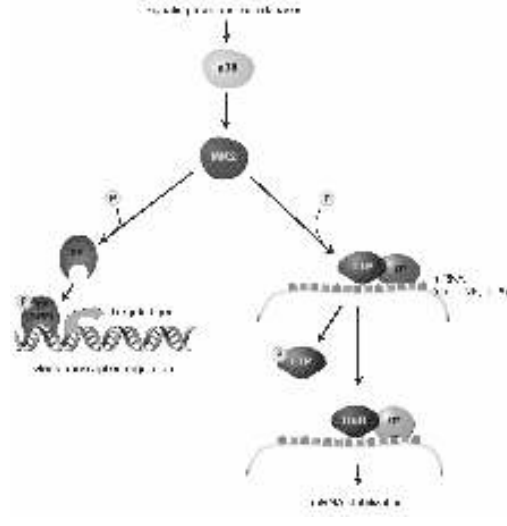


Figure 1. Regulation of inflammation by MK2 at molecular level.

Upstream signaling kinase activates $p38^{MAPK}$-MK2 axis and activated MK2 regulates inflammation by two means. First, activated MK2 phosphorylates Tristetraprolin (TTP) which, along with its interaction partner (IP) destabilizes/degrades mRNA, when in un-phosphorylated form. Phosphorylated TTP gets displaced from adenylate-uridylate-rich element (ARE) of mRNA by human antigen R (HuR) and its IP resulting stabilization of mRNA prolonging protein translation. Second, through transcriptional regulation of the genes involved in inflammation via phosphorylation of transcriptional factors (TF, e. g. SRF & HSF1).

Another constitutively ARE binding protein human antigen R (HuR) functions in a competitive manner in exchange with TTP, upon phosphorylation affinity of TTP reduces which leads to its replacement by the HuR, initiating the translation and vice versa [46]. Apart from TNF, MK2 also regulate the stability of other mRNA such as COX-2, GM-CSF, INF-γ, urokinase plasminogen activator (uPA) and IL-1, -4, -6, -8 mRNA at the post transcriptional level [41, 47-51].

MK2 also regulates inflammation at the transcriptional level via NF-κB. Activated MK2-HSP27 retains $p38^{MAPK}$ in cytoplasm thus preventing it from phosphorylating nuclear MSK1 and hence the nuclear export of NF-κB is prevented, resulting in transcription of NF-κB regulated genes including the one involved in inflammation [52]. MK2 phosphorylates and inhibits the activity of heat shock transcription factor 1 (HSF1), which represses cytokine transcription [53].

MK2 in inflammatory condition related diseases

Regulation of the inflammatory response by MK2 was evident when MK2 knockout mice showed increased stress resistance and survival due to reduced biosynthesis of TNF-α at post-transcriptional level upon lipopolysaccharide induced endotoxic shock [42]. Hence, it's obvious that MK2 may play a critical role in inflammatory condition related disorders. Here I discussed the studies involving experimental models implicating the importance of MK2 (Figure 2). In the disease model of collagen-induced arthritis, MK2-deficient mice shows resistant to the disease [54] while MK2 was reported in modulating key biological pathways associated with and contributing to joint structural deterioration in osteoarthritis [55].

Figure 2. The role of MK2 in the inflammatory condition associated pathophysiology.

The outer circle represents diseases having inflammatory axis where MK2 play a role and inner circle represents physiological systems related to those disease conditions.

Gene deletion of MK2 protects against cerulein-induced pancreatitis by inhibiting TNF-α and IL-6 [56]. MK2 deficiency inhibits inflammatory responses in the inflammatory skin diseases [57] including systemic inflammation [58]. Tumerogenesis is well associated with inflammation, like wise MK2 was reported to regulate the early stages of skin tumor promotion through inflammatory promotion of papilloma formation [59]. Furthermore, the role inflammatory mediators have been implicated in the pathogenesis of cardiovascular disorders (CVD) including atherosclerosis, type-2 diabetes, obesity-related metabolic dysfunction, neointimal hyperplasia and endothelial dysfunction. Systemic deficiency of the MK2 was shown to reduce atherosclerosis in hypercholesterolemic mice [27] and angiotensin II-induced vascular inflammation was ameliorated by MK2 inhibition [60]. Cardiac oxidative stress, inflammation and remodeling were mediated via modulation of $p38^{MAPK}$- MK2-NF-κB signaling pathways after streptozotocin-induced diabetes mellitus [61]. Same axis was shown to regulate the level of negative feedback in the NF- κB pathway in airway inflammation [52]. Neuroinflammation associated with Alzheimer disease [62] and LPS-induced inflammatory bone loss [63] were shown to be prevented in MK2 deficiency. Inhibition of MK2 reduces inflammation in dextran sulfate-induced colitis [64] and postoperative ileus in mice [65]. In addition, MK2 also contributes to Shiga toxin-induced inflammatory response [66] and Clostridium difficile-associated inflammation [67] suggesting that MK2 also plays role in these infectious/septic diseases. The role of mitogen-activated protein kinases, including MK2 is described very excellently in recent reviews [68, 69].

MK2 inhibition over $p38^{MAPK}$

Previous strategies in developing selective $p38^{MAPK}$ inhibitors have proved ineffective due to many reasons. Several $p38^{MAPK}$ inhibitors showing promising results in preclinical phase failed in clinical trials due to side effects related to central nervous system, hepatotoxicity and induction of kinases that can take over the role of $p38^{MAPK}$ [70, 71]. Moreover $p38^{MAPK}$ is not an attractive target for the development of a drug for several reasons. First, it is involved in feedback regulation of upstream kinase [72] which are involved in other inflammatory pathways, over activation of which may pose other toxicity related problems [73]. Second, it has about one hundred targets which make signaling extremely diverse and affecting pathways out of therapeutic interest [74]. Third, $p38^{MAPK}$ activates anti-inflammatory pathways by inducing transcription of the mitogen-activated protein kinase phosphatase DUSP1 and the anti-inflammatory cytokine interleukin 10 [75] and by suppression of prostaglandins [76].

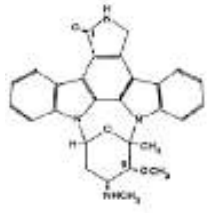
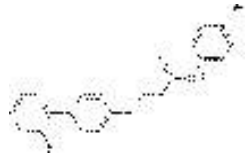
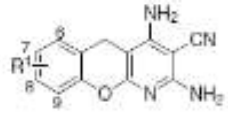
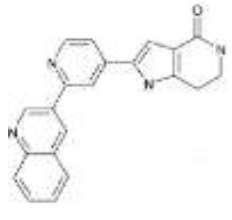
Recently it has been observed that $p38^{MAPK}$ inhibition, not MK2 inhibition enhanced

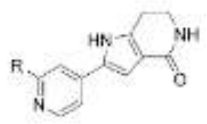
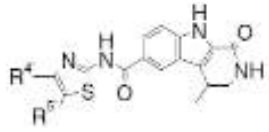
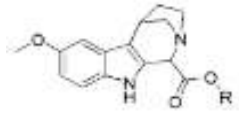
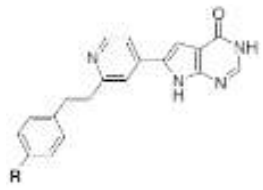
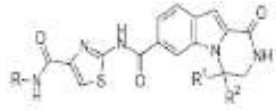
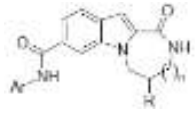
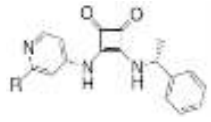
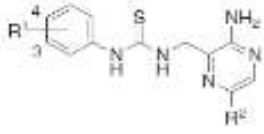
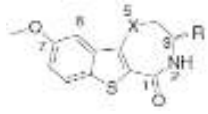
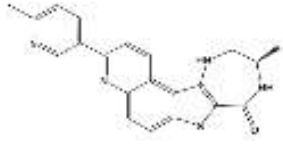
secretion of inflammatory chemokines upon TNF-α stimulation from the cells pointing to the lack of efficacy of $p38^{MAPK}$ inhibition [77]. Mice lacking MK2 produced significantly less cytokines, especially TNFα in response to lipopolysaccharide compared to control littermates indicating MK2 as an essential component of the inflammatory response [35]. Moreover, lack of MK2 has been proven to be beneficial in various pathological conditions as discussed earlier. These findings make MK2 a very attractive target for the pharmacological inhibition in the inflammation and related pathological conditions.

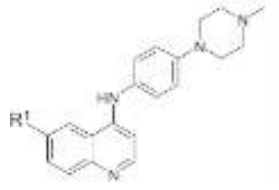
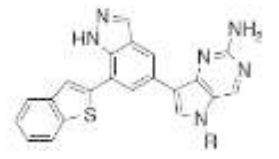
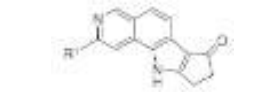
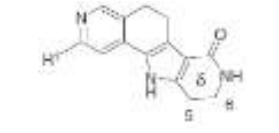
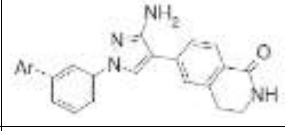
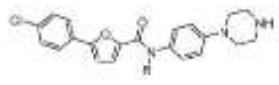
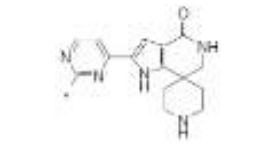
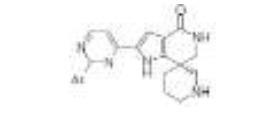
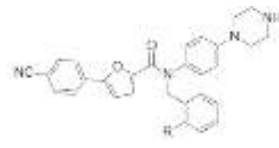
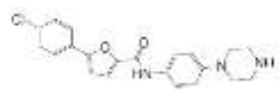
MK2 inhibitors in the development

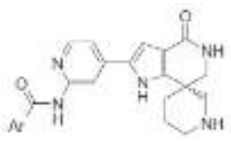
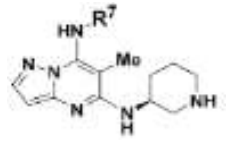
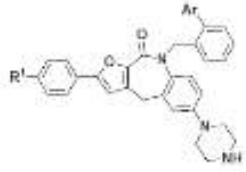
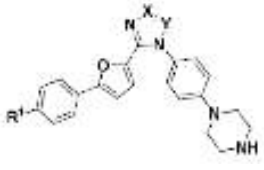
During the past decade, many MK2 inhibitors are generated (Table 1) and tested for their potency, cellular efficacy and *in vivo* effects. Of these selected inhibitors are discussed here group wise.

Table 1. List of MK2 inhibitor.

Compound/Class	Structure	Best analogue	MK2 IC_{50} (nM)	Mechanism of action	Remarks	Developer	Ref.
Natural products		Staurosporine	180	ATP competitive	Study included compounds with wide structural differences		[99]
CMPD		CMPD1	330	Non-ATP competitive	Inhibits a splice variant of MK2 (MK2a) through substrate specific p38α inhibition	Boehringer Ingelheim	[107]
Aminocyanopyridine		Compound 2a	130	ATP competitive	Orally active but with unacceptable therapeutic profile	Pfizer	[12, 79]
Pyrimidylpyrrole		Compound 1	8.5	ATP competitive		Bayer Schering	[106]

Pyrrolopyridine (PH-089)		Compound 23	126	ATP competitive		Pfizer	[103]
Carbolin		Compound 83	44	ATP competitive		Boehringer Ingelheim	[86]
β-carboline carboxylic acids		Compound 96	>1000	ATP competitive	A prodrug, active *in vivo*	Pfizer	[83]
Pyrrolo-pyrimidones		Compound 16	51		Modest selectivity	Novartis	[108]
Pyrazinoindolone		Compound 32	2		Good pharmacokinetic properties and specificity	Boehringer Ingelheim	[87]
Indole		Compound 25a	29			Boehringer Ingelheim	[109]
Squarate based		Compound 42	>1000		Potency remained low	Wyeth (Now part of Pfizer)	[102]
Thioureas		Compound 12f	15		Active *in vivo*	Merck	[110]
Benzothiophene		Compound 29 and 31	5			Pfizer	[80, 81]
		PF-3644022	5.2	ATP competitive	Large predicted human dose, Acute hepatotoxicity in dog and monkey	Pfizer	[82]

Anilinophenylquinoline		Compound 2e	400	Non-ATP competitive	Mixed inhibition with dominating element of uncompetitive inhibition	AstraZeneca	[111]
Diaminopyrimidine		Compound 31a	19	ATP competitive	Development discontinued	Abbott	[84]
Tetracyclic		Compound 14F	160		Orally bioavailable	Novartis	[91]
		Compound 13E	50		Good oral efficacy, well tolerated in mice, Inhibited other 14 kinase	Novartis	[92]
Aminopyrazole		Compound 14e	61		Orally active	Novartis	[112]
Furan carboxyamide		Compound 25	110	Non-ATP competitive		Merck	[93]
Spiro-δ-lactam		Compound 5b	NA*	ATP competitive	Active *in vivo* but lacks oral bioavailability, EC_{50}=4 nM	Merck	[88]
Spiro-3-piperidyl		Compound (S)-23 and (S)-25	NA	ATP competitive	Orally bioavailable	Merck	[89]
Phenyl furany amide		Compound 28	8	Non-ATP competitive		Merck	[113]
Tetracyclic azepine and Oxazocine		Compound 29	2.9	Non-ATP competitive		Merck	[94]

Pyrrolopiperidone		Compound 6d.S1	NA	ATP competitive		Merck	[90]
Pyrazolopyrimidine		Compound (S)-44	130	ATP competitive	Selective, Good ADME with oral bioavailability, active in vivo	Teijin	[85]
Tricyclic lactams		Compound 2S	1.9	Non-ATP competitive		Merck	[95]
Imidazole/ Triazole		Compund 13a	22.5	Non-ATP competitive	Improved permeability and *in vivo* availability compared to tricyclic lactams [95]	Merck	[96]

* NA: not available

Synthetic compounds

Following the failure of $p38^{MAPK}$ inhibitors as anti-inflammatory drugs in clinical trials due to its unacceptable safety profile, pharmaceutical industry has turned their focus towards potential MK2 inhibitors. Since MK2 is a downstream target of $p38^{MAPK}$ involved in less complex signal transduction than $p38^{MAPK}$ and mainly responsible for pro-inflammatory cytokine-regulation [78], its inhibition could give a better and acceptable safety profile.

A first small molecule inhibitor of MK2 was reported by *Anderson et al* [79] which was again synthesized by *Davis et al* to study in Werner syndrome cells but result remain unacceptable [12]. A benzothiophene class of compounds was developed and evaluated for selectivity and potency [80] but they were not up to expectation. So the further efforts were taken to bring improvements in kinase selectivity and cell potency [81]. One of these benzothiophene inhibitors of MK2: PF-3644022 was reported to display good pharmacokinetic parameters in rats having orally efficacy in both the rat acute LPS-induced TNF-α model and the chronic streptococcal cell wall-induced arthritis model [82]. The future of PF-3644022 remains uncertain as its projected human dose would be large and acute hepatotoxicity was observed in dogs and monkeys, although it was well tolerated in rats [82]. Another structural class of compounds as MK2 inhibitor were

reported through structure–activity relationship study, which were less potent than former, but has better selectivity against MK2 [83]. Due to difficulties in improving oral efficacy, cellular and enzymatic potency further development of diaminopyrimidine class of inhibitors was discontinued [84]. Pyrazolo pyrimidine class of derivatives showed excellent selectivity and good ADME accompanied with *in vitro* cellular potency as anti-TNF-α agents and *in vivo* efficacy in a mouse model of endotoxin shock [85]. The potency of a carbolin based MK2 inhibitors [86] was improved by transposing the indole nitrogen from the carbolin scaffold to the corresponding pyrazinoindolone scaffold [87]. Structure based lead identification of a class of spiro-δ- lactam MK2 inhibitor produced compounds which were active *in vivo* with good selectivity while it had a major issue of lack of oral bioavailability [88]. The issue of oral bioavailability was addressed by moving the position of the piperidyl nitrogen in structure so as to generate orally available compounds [89]. To further improve the cell based potency of these compounds the structural alterations were performed using computation chemistry [90]. A series of tetracyclic MK2 inhibitors were reported in two parts [91, 92], in the first part a tetracyclic ketone proved to be orally bioavailable with good selectivity against a panel of kinases in an *in vivo* study pointing to MK2 specific actions. In the second part, two orally active MK2 inhibitor series the spirocyclopropanes and the spiroazetidine were discovered. The spiroazetidines showed very potent MK2 inhibition but they generally suffer from low oral absorption and high clearance. The spirocyclopropanes on the other hand were less potent, however they display better absorption and moderate clearance *in vivo* [91, 92].

The non-ATP-competitive MK2 inhibitors based on a furan-2-carboxyamide scaffold was discovered through high throughput screening using the affinity selection-mass spectrometry-based Automated Ligand Identification System platform [93] where compound 25 showed promising results by inhibiting secretion of pro-inflammatory cytokines and dose dependently inhibiting TNF-α, IL-6 and matrixmetalloprotease. Moreover, it also showed excellent kinase selectivity, drug metabolism and pharmacokinetics [93]. Recently, conformationally restricted tetracycles over non-cyclized compound were reported to be a very potent with regards to MK2 inhibition (IC50) and/or cellular activity from same laboratory [94]. Further efforts were taken to improve profile of these compounds by confirmation changes and substitutions [95, 96].

Peptides

Protein kinases are often maintained inactive by an autoinhibitory loop/region masking catalytic activity [97]. A 14 amino acid peptide was derived from the autoinhibitory domain of MK2 which inhibited its kinase activity in a concentration dependent manner [98]. By other means of inhibition pseudo substrate peptides were derived which has mutated phosphorylation site. One of them, peptide

P3 showed potent inhibition of kinase activity [99]. Unfortunately, these peptides were not specific as it inhibited other kinase as well like protein kinase A and C (PKA and PKC).

Discovery of protein uptake by the cells growing in tissue culture [8, 11] raised possibilities in delivering therapeutics as a cargo. *Brugnano et al* took the advantage of this discovery in generating cell penetrating peptides (CPPs) linked with MK2 inhibitory peptide [15], however its efficacy and *in vitro* therapeutic functionality were not promising. It was revealed that apart from delivering peptides CPPs has an independent biological activity, which may be of major limitation in targeted delivery. *Lopes et al* developed cell permeable MK2 inhibitor peptide MK2i based on peptide P3 [99] which suppressed fibrotic response [16] but selectivity and efficacy were not studied. Furthermore MK2i was shown to inhibit intimal hyperplasia in a human saphenous vein organ culture model [17]. In order to improve specificity and reduce toxicity a peptide called MMI-0100 linked with CPP was generated which showed potential for inhibition of abdominal adhesion during surgical procedures, while the peptide was not 100% specific but there were no obvious *in vivo* side effects [100].

Natural products

Natural product as a MK2 inhibitor has also been explored. Secondary metabolite alkaloids from microbial sources staurosporine (from Streptomyces sp.) and K-252a (from Nocurdiopsis sp.) and other natural products were reported to inhibit protein serine threonin kinase HSP25 kinase or MK2 in ATP competitive manner [99]. Meroterpenoid inhibitors of MK2 (+)-Makassaric acid and (+)-subersic acid were isolated from Indonesian marine sponge Acanthodendrilla sp. [13] followed by chemical synthesis of (+)-Makassaric acid [101].

Challenges

The decade long efforts to develop MK2 inhibitor have not given any outcome yet. These efforts are on the back foot due to several challenges. More than 500 kinases have been identified which binds to ATP in an almost similar way to attain functional status. Owing to high cellular concentration of ATP (up to 5 mM) and comparatively high ATP binding affinities of protein kinase it's difficult to develop an ATP competitive inhibitor with sufficient selectivity and cellular activity. Competition with high cellular concentrations of ATP dramatically reduces the cellular potency of an ATP competitive inhibitor.

The application of structure based drug design is limited by the availability of the low resolution crystal structure of MK2 [80, 102-105]. In addition, narrow and deep ATP-binding pocket in MK2 as reported by solving MK2 structures further increases difficulties [80, 81, 103, 106]. The alternative to these problems would be to develop a non-ATP competitive inhibitor, which may improve biochemical

efficiency through a non-competitive and selective binding mode.

Conclusion

Overall, the involvement of MK2 in various pathological conditions associated with inflammatory conditions indicates a high therapeutic potential of MK2 inhibition. Till date, development MK2 inhibitor is in its infancy and substantial work needs to be done as none of these MK2 inhibitors made it to clinical trial. The development of small molecule MK2 inhibitor will provide a better and safe therapeutic option in future.

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