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REVIEW ARTICLE

BIOCHEMISTRY

P38 MAPK ACTIVATION IN MYOCARDIAL ISCHAEMIA

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ABSTRACT

Ischaemic heart disease remains, and is likely to continue to be, the leading life threatening disease around the world. Signaling pathways have become more interesting as novel therapeutic targets in ischaemic heart disease. However, one needs to be very careful in picking the therapeutic target as one signaling molecule can activate and also cross-talk with other kinases. The activation of the p38-MAPK during myocardial ischaemia aggravates lethal injury. Recent evidences suggested the mechanism of p38-MAPK activation may differ by circumstances. Determining the precise mechanisms is crucial since it may allow prevention of the detrimental, but not the beneficial, and lead to the identification of the relevant downstream signals. Therefore, p38 MAPK may be a viable clinical target and form the basis of future studies designed to further dissect the signaling pathways and discover the downstream substrates will become hopes as a new frontier of therapeutic approach in ischaemic heart diseases.

KEY WORDS

p38 MAPK; Myocardial Ischaemia; Autophosphorylation; Transphosphorylation

INTRODUCTION

Cardiovascular diseases (CVD), principally heart disease and stroke, are a leading cause of global morbidity and mortality. The World Health Organization (WHO) statistic report 2008 predicted that, although non-communicable diseases are considered the leading killers worldwide, currently, ischemic heart disease and cerebrovascular disease (stroke) will become the 2 diseases resulting in the majority of deaths in 2030 [1]. Most seriously, ischemic heart disease is still ranked on the top of the mortality, by cause, from 2004 to 2030. Although the annual mortality from this disease is decreasing in developed countries [2] it is increasing in the more populace developing countries [3]. Ischaemic heart disease, otherwise known as coronary artery disease, is a condition that affects the supply of blood to the heart, which is essential for proper functioning of the heart. This may eventually result in a portion of the heart being suddenly deprived of its blood supply leading to the death of that area of heart tissue, resulting in a heart attack or acute myocardial infarction. Currently, the most efficient method of reducing mortality in such patients is to achieve rapid reperfusion by lysis or mechanical disruption of the occlusive coronary thrombus and plaque. The mortality from acute myocardial infarction under these circumstances is inversely related to the amount of myocardial salvage achieved by reperfusion,[4] so any intervention that slow the rate of ischaemic necrosis are likely to save many lives.[5] There are many evidences demonstrated that the activation of p38 MAPK that occurs during prolonged ischaemia[6] accelerates injury. The information achieved from p38 MAPK inhibition by pharmacological inhibitors[7-32] or genetic modified to knock down p38 MAPK or its downstream effectors [33-35] means slows the rate of infarction/death. Although this evidence is based on animal data it seems likely similar mechanisms operate in the human heart since p38 MAPK is identically activated by ischaemia[36, 37] and early clinical trials indicate a potential benefit. Thus, in theory at least, inhibitors of p38 MAPK have therapeutic potential in ischemic heart disease[38].

GENERAL BACKGROUND OF P38 MAPK

The p38 MAPK, (also known as CSBP, mHOG1, RK, and SAPK2)[39, 40] is a family of serine/threonine protein kinases that plays an important role in cellular responses to external stress signaling and also function in many cellular processes including inflammation, cell differentiation, cell growth and death. The human p38 MAPK was originally isolated as a 38-kDa protein rapidly tyrosine phosphorylated in response to lipopolysaccharide (LPS)-stimulation in human monocytes [40] . Moreover, it was also identified as the target of a pyridinyl imidazole drug that blocked production of tumour necrosis factor-α (TNFα) and as such was called cytokine-suppressive anti-inflammatory drug-binding protein or CSBP [40].

There are 4 isoforms of p38 MAPK that have been identified including p38 α, β, γ, and δ. Of these, p38α (or MAPK14) is the best characterized and perhaps the most physiologically relevant kinase involved in inflammatory responses, and found that has more than 70% identity of p38β (or MAPK 11)[41, 42]. Both α and β are ubiquitously expressed in several tissues, but are thought to have different functions [41, 42]. Moreover, these 2 isoforms are sensitive to p38 MAPK inhibition by a pyridinyl imidazole inhibitor. p38γ (also known as SAPK3 and MAPK12) is largely expressed in skeletal muscle, whereas p38δ (also known as SAPK4 or MAPK13) [41-45] is expressed more widely in several adult tissues and during development. Sequence comparisons revealed that each p38 isoform has more

than 69% identity within this group, but only 40 to 45% to the other MAP kinase family members [46]. These 2 isoform are different from α and β in term of sensitivity to pyridinyl imidazole inhibitor, as γ and δ found to insensitive to kinase inhibition by this family of inhibitor.

ACTIVATION OF P38 MAPK AND ITS ACTIVITY

Similar to other MAPKs pathway, p38 MAPK is activated as a result of phosphorylation at specific sites by upstream family of MAPKKK and MAPKK, consequently. p38 activation in response to a variety of extracellular stimuli can be observed by the diverse range of MAP kinase kinase kinases (MAP3Ks) that participate in p38 activation. These include TAK1 [47], ASK1/MAPKKK5 [48], DLK/MUK/ZPK [49, 50], and MEKK4 [49, 51, 52]. Subsequently activation downstream of MAP3Ks is a selective activation of MAP kinase kinases (MKKs) or MKKs, which lie upstream of p38 MAPK. This MKKs are selectively dual phosphorylated p38 MAPK at specific phosphorylation sites, Thr^{180} and Tyr^{182}. There are two main MKKs that are known to activate p38, MKK3 and MKK6 (Figure 1).

MKK induced dual phosphorylation of p38 at Thr^{180} and Tyr^{182} is thought to cause the activation loop to refold [53] and move out of the peptide-binding channel. This movement is then thought to exert a "crank-handle" effect on the overall tertiary structure of the kinase reorientating, which enabling the cooperation necessary for ATP binding [54]. In support of this model Diskin et al., based on a gain of function mutagenesis screen of the yeast p38 homologue, demonstrated p38 MAPK activation can also be achieved in the absence of the dual phosphorylation of Thr^{180} and Tyr^{182} by mutations that similarly disrupt their hydrophobic environment [55].

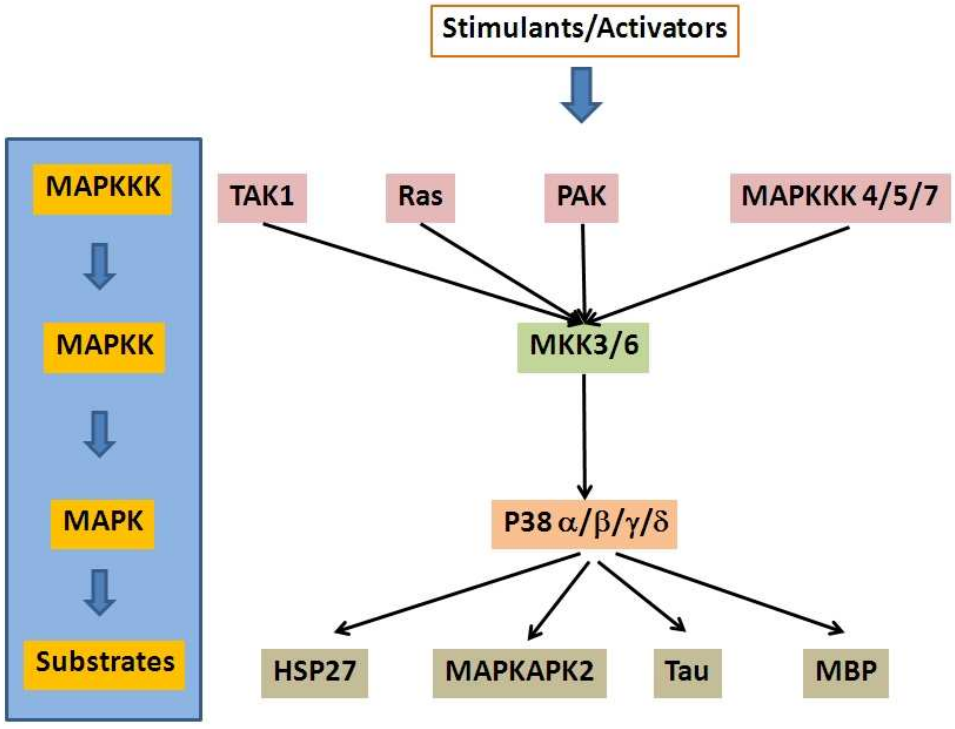

Figure 1
p38 MAPK activators and substrates.

When p38 MAPK is in the non-dual phosphorylated, inactive conformation, the loop is thought to reside in the peptide-binding channel that lies in the cleft between amino- and carboxy-terminal lobes of the kinase. In addition, in p38 MAPK, in contrast to p42/44-MAPK, there is a misalignment of these lobes, that prevents the co-operation between Lys[53], in the N-terminal lobe, and Asp[168], in the C-terminal lobe, imperative to the binding and stabilization of the alpha-phosphate group and adjacent ribose of ATP, respectively [56]. Therefore, it is widely thought that the nondual phospho- form of p38 is inactive as a result of steric obstruction of the peptide-binding channel and low ATP affinity [56].

After p38 MAPK dual phosphorylation, the active form of p38 MAPK can access to ATP and use its kinase activity to phosphorylated specific downstream substrates. The MAPKs have a substrate preference, but not an absolute requirements, for sites containing a serine or threonine followed by a proline residue [57]. There are 2 distinct types of p38 MAPK downstream substrate including protein kinases and transcription factors. MAP kinase-activated protein kinase 2 (MAPAP-K2 or M2) was the first identified p38 MAPK substrate [58, 59] (Figure 1). This substrate has kinase activity itself and is shown to phosphorylate various substrates including small heat shock protein 27 (HSP27) [60], lymphocyte-specific protein 1 (LSP1) [61], cAMP responsive element-binding protein (CREB) [62], Serum Responsive Factor (SRF) [63] and tyrosine hydroxylase [64].

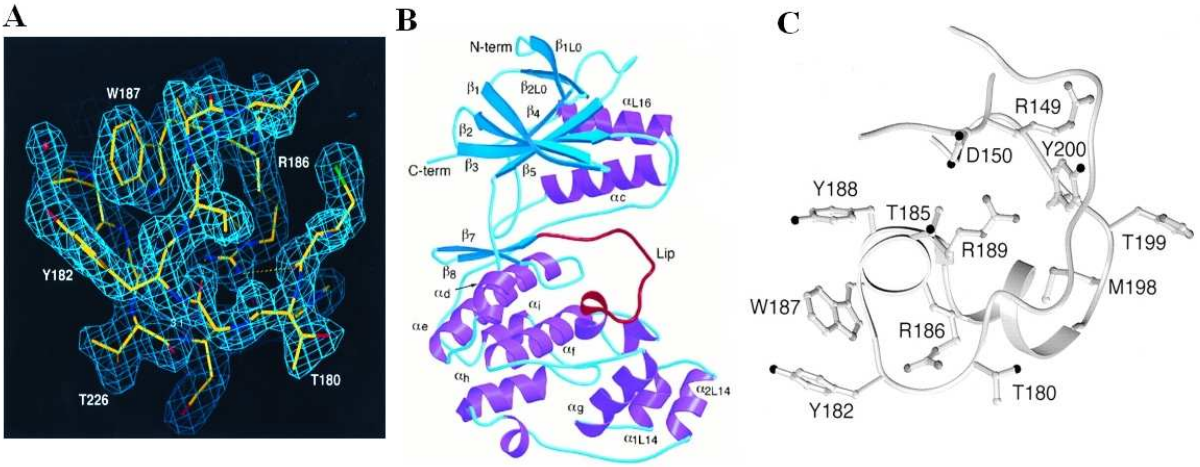

Figure 2

Structure of p38 MAPK. A, electron density around the phosphorylation sites Thr[180] and Y[182]. B, Ribbon diagram of p38. C, the specific pocket and phosphorylation lip region of p38. This figure modified from Wang et al, 1997.[65]

MECHANISMS OF P38 MAPK

Transphosphorylation

External stimuli initiate the activation of the serine/threonine MAP kinase kinase kinases (MAPKKKs) that are upstream of p38. Theses kinases phosphorylate and activate the dual-specificity kinases MAPKK (MKK), which in turn phosphorylate p38 on Thr[180] and Tyr[182] (Figure 3)

Several upstream MAPKKs (MKK) have been identified from *in vitro* analysis, including MKK3 and MKK6 [66, 67]. Mice lacking both MKK3 and MKK6 are not viable, dying in midgestation with defects in the placenta and

the embryonic vasculature [68] Moreover, Deacon and Blank [69] showed that MKK4, which is a MAPKK involved in JNK activation, can also activate p38 *in vitro*.. However, p38 activation is not limited to this traditional phospho-relay signaling cascade. Since SB203580, the most widely used p38 kinase inhibitor occupies the catalytic site, without inhibiting upstream MKK, it should only inhibit the phosphorylation events downstream of p38 without inhibiting the dual-phosphorylation of p38 itself [70]. Nonetheless the literature is replete with examples of SB203580, and structurally related compounds [71], inhibiting the dual phosphorylation of p38 MAPK [72-77]. Two possible explanations of these findings are p38 is able to autophosphorylation its activation loop or the inhibitory effect of SB203580 is on a kinase upstream of p38 involved in its activation by transphosphorylation.

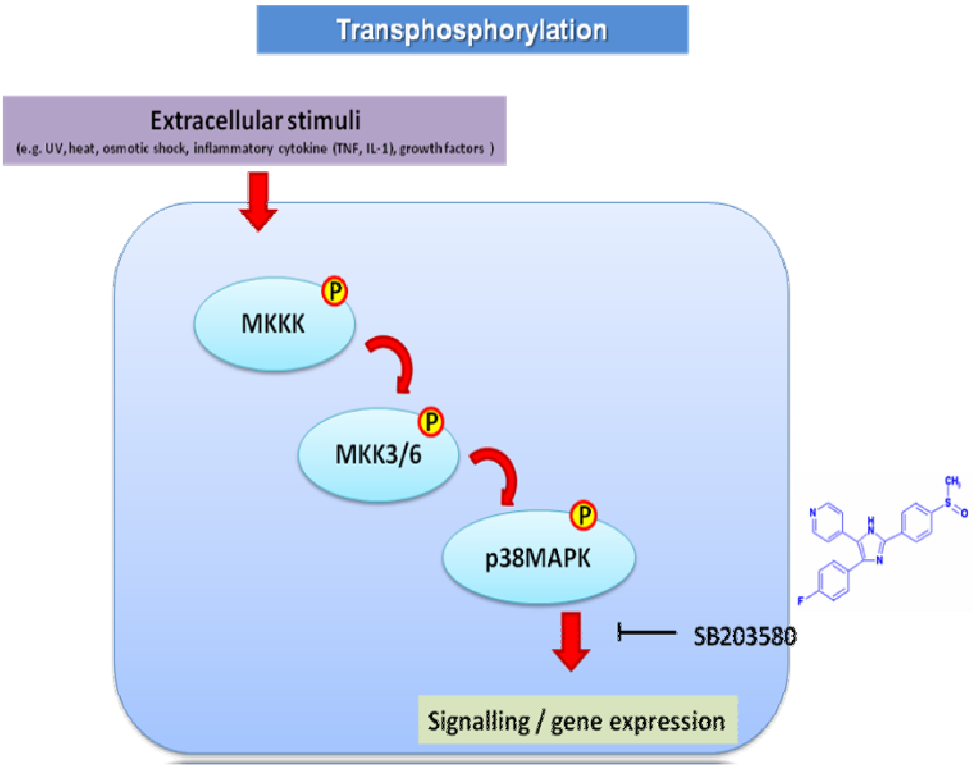

Figure 3
Schematic representation of the transphosphorylation mechanism of p38 activation.

Autophosphorylation

It has long been known that, as with other MAPKs, p38 can autophosphorylate *in vitro* to a limited extent [78, 79]. The degree of autophosphorylation is influenced by the length of the activation loop containing the TGY phosphorylation motif and the identity of the non-phosphorylated intervening residue (for example, autophosphorylation is more likely to occur if there is an intervening aspartic acid residue rather than a glycine residue) [78, 79].

Ge et al reported the MKK independent activation of p38 MAPK mediated by the interaction of scaffolding protein TAB1 [80] (Figure 4). TAB1, or transforming growth factor-β-activated protein kinase-1 (TAK1) binding protein-1, is a scaffold protein known to associate with TAK1 [81] and facilitate TAK1 autophosphorylation [82]. TAK1 is also an upstream kinase of MKK3 and MKK6. Through a comprehensive series of experiments in HEK293 cells, Ge et al demonstrated a similar functional interaction

with p38 MAPK [80]. For example the ectopic expression of TAB1 caused the dual phosphorylation of wild-type p38 MAPK, but not of p38 mutants rendered kinase dead by substitution of the critical Lys^{53} or Asp^{168} residues. This was despite the fact that TAB1 still interacted with these mutants on the basis of co-immunoprecipitation and that the mutants could be transphosphorylated in the presence of constitutively activated MKK3 or MKK6 [80]. Reciprocally, the TAB1-induced activation of wild-type p38 MAPK could not be prevented by co-expression of dominant negative forms of MKK3, MKK6 and/or TAK1, but could be prevented by SB203580. When differentially tagged wild-type and kinase dead forms of p38 MAPK were co-incubated with TAB1 only the wildtype form was dual phosphorylated implying that the observations were the result of true, intramolecular, autophosphorylation rather than one p38 MAPK molecule transphosphorylating another [80]. The activation of by the autophosphorylation of p38α after interaction with TAB1 although there is an indication that TAB1-dependent p38 phosphorylation occurs in LPS, TNF, and CpG treated B cell lines.

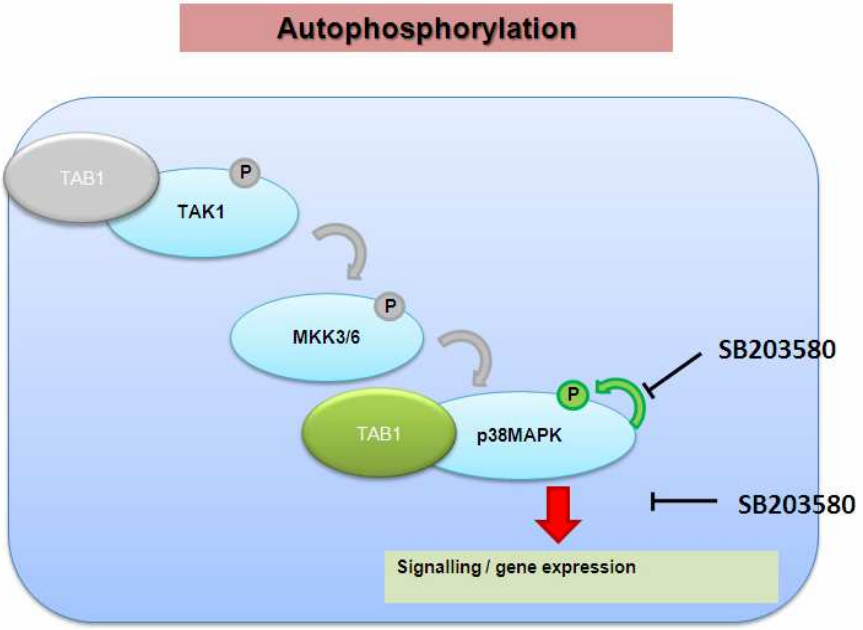

Figure 4
Schematic representation of the Autophosphorylation mechanism of p38 activation. TAB1 association with p38, induces p38 autophosphorylation in an SB-sensitive manner.

Negative Feedback Autophosphorylation

In 2003, one year after Ge et al. reported TAB1 mediated autophosphorylation of p38, Cohen's group independently identified TAB1 as a p38 interacting protein by yeast-two hybrid screening [83]. This finding made the interpretation of TAB1-p38 interaction and mediation of p38 activation more complicated as they showed that TAB1 is also phosphorylated on Ser^{423}, Tyr^{431}, and Ser^{438}. Moreover, p38 induced phosphorylation of TAB1 on Ser^{423} and Tyr^{431} which was inhibited by SB203580 [83]. Inhibition of p38 mediated phosphorylation of TAB1 (Ser^{423}/Tyr^{431}) by SB203580 enhanced the activity of TAK1, which then activated p38 via MKK3 and MKK6. These findings proposed a feedback control mechanism of TAK1 activity, whereby p38 activity inhibits TAK1, through the phosphorylation of TAB [83] (Figure 5). Inhibition of p38 activity by SB203580 can abolish this feedback control and cause the

activation of the parallel JNK pathway and consequently IKK [83].

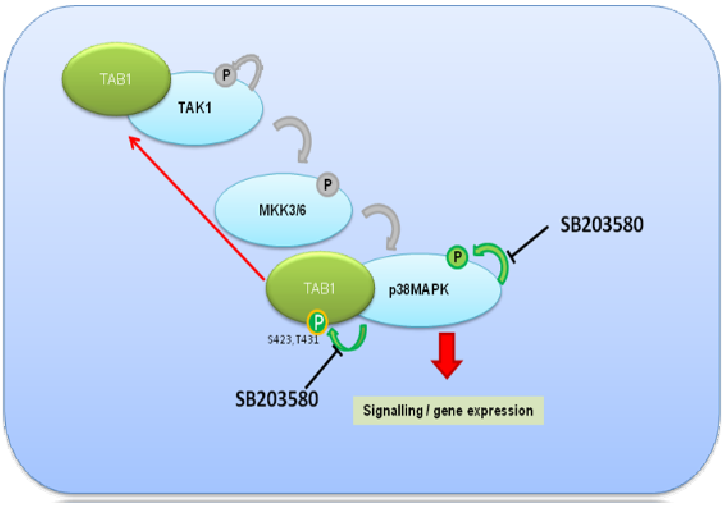

Figure 5
Schematic representation of the Negative Feed Back Autophosphorylation mechanism of p38 activation.

TAB1 association with p38 induces p38 autophosphorylation in an SB-sensitive manner. This interaction modulating p38-MAPK activity directly, it was involved in negative feedback control of activation through TAK1. The phosphorylation of Ser^{423} and Tyr^{431} of TAB1 by p38-MAPK markedly diminished the ability of TAB1 to activate TAK1 as a consequence the activation of MKK6 was diminished and therefore the dual phosphorylation of p38-MAPK reduced.

p38 MAPK, MYCARDIAL ISCHAEMIA, AND ISCHAEMIC HEART DISEASES

Myocardial ischaemia is a potent stimulant of p38 MAPK activation, which is an important pro-apoptotic kinase in cardiac myocytes [84]. There is increasing evidence from preclinical investigations that inhibition of p38 during prolonged ischaemia slows the rate of infarction/death and inhibits the production of inflammatory cytokines, such as TNF-α, IL-1, and IL-8, which inturn aggravate ischaemic injury [56, 70].

Clinically, prompt reperfusion following coronary artery occlusion remains the most effective intervention to re-establish arterial patency and reduce ischaemic myocardial injury [84]. However, reperfusion can re-activate p38 MAPK, perhaps in response to stimuli such as ROS and osmotic stress [84]. Although this field of research remains controversial, compelling evidences support a causative role of p38 in myocardial injury and dysfunction following ischaemia and reperfusion [33, 84-86].

Many studies have elucidated the mechanisms, such as apoptosis and inflammation, through which p38 activation might contribute to ischaemic injury [84]. Bogoyevitch et al, first demonstrated that p38 α and β isoforms are activated in response to ischaemia and reperfusion in the heart [87]. Many studies using ectopic gene expression indicated that the α isoform is implicated in cardiomyocyte apoptosis and this isoform alone is sufficient to cause cell death following ischaemia [19, 24, 87]. In addition, the studies using a selective p38 inhibitor, SB203580, highlighted the pro-apoptotic role of p38 in cardiomyocytes during ischaemic injury [87, 88]. Moreover, there is overwhelming evidence that inhibition of p38 using pharmacological inhibitors [8, 19, 21, 23-25, 27], or genetic means [33, 35] slows the rate of infarction.

Although this evidence is based on animal data it seems likely similar mechanisms operate in the human heart since p38 MAPK is identically activated by ischaemia [36, 37].

Thus, superficially at least, inhibitors of p38 MAPK have therapeutic potential in ischaemic heart disease [38].

However, a beneficial effect can also follow p38 activation, since under many circumstances its activation leads to protection rather than to injury [89-92]. This particularly seems to be the case when myocardial p38 MAPK activation occurs as a consequence of a sub-lethal stress that precedes lethal myocardial ischaemia. For example, in many studies [18, 21, 23] p38 MAPK inhibition during a "conditioning" sub-lethal stress abolishes subsequent ischaemic protection. However in these studies the same inhibitor, at the same concentration, reduces injury if present solely during lethal ischaemic injury [18, 21, 23, 27, 93]. The cause of these apparent paradoxical observations is complex but may relate to an attenuation of p38 MAPK activation during lethal ischaemia by prior transient activation [18, 21, 23, 27, 93, 94]Furthermore very recently pre-ischaemic activation of p38 MAPK by transgenic overexpression of an upstream kinase led to similar protection [89]. However p38 MAPK content was reduced in transgenic hearts possibly resulting in reduced active p38 MAPK during ischaemia and complicating interpretation [89]. Whatever the exact underlying mechanism there is ample evidence from the cardiac, [18, 21, 23, 89-92] as well as from others [95, 96] research fields that p38 MAPK activation can have beneficial consequences. However it is also incontrovertible that restricting p38 MAPK inhibition to the activation that accompanies lethal myocardial ischaemia reduces infarction [8, 11, 18, 19, 21, 23-25, 27, 33, 35, 85]. Taken together these observations compel a greater understanding of the mechanisms and targets of p38 MAPK activation. Without such an understanding there is a danger of repeating the previous failings made when clinical trials, in the related area of anti-TNF therapy, proceeded without due appreciation of contrary data [95, 97, 98]. This has become especially pertinent since a phase 2 clinical trial of p38 MAPK inhibition during acute coronary syndromes has just been completed with a positive result based on a surrogate primary outcome [38, 99]. Again reminiscent of the early experience with anti-TNF therapy [100].

MECHANISMS OF P38 MAPK DURING MYOCARDIAL ISCHAEMIA

There are many studies investigated the actually mechanism of p38 MAPK activation occur during myocardial ischaemia. According to studies using pharmacological inhibitor SB203580, there are numerous examples where this has been observed (often inadvertently) during myocardial ischaemia[7, 9-11, 28, 31, 101-103]. Thus unlike most stresses, myocardial ischaemia seems to reproducibly cause an SB-sensitive form of p38-MAPK dual phosphorylation. Two mutually exclusive explanations for this observation are that p38-MAPK is able to **AUTOPHOSPHORYLATION** its activation loop or that SB203580 inhibits a kinase upstream of p38-MAPK involved in its activation, by **TRANSPHOSPHORYLATION**, during ischaemia.

In attempt to prove the mechanism of p38 MAPK activation during myocardial ischaemia is achieved by transphosphorylation, Jacquet et al showed that RIP2 Kinase, another upstream kinase of p38 MAPK and known to be inhibited byp38 MAPK inhibitor, SB203580[104], does not contribute to p38 MAPK activation in response to myocardial ischaemia[105]. Although the hypothesis of RIP2 mediated transphosphorylation of p38 in response to ischaemia was not proved, the hypothesis of transphosphorylation p38 MAPK by SB-sensitive upstream kinases is still viable. Thus identification of other cardiac kinases binding to SB203580 is still important for future studies.

Recently Kumphune et al, showed the knock-in mice, expressing specific inhibitor resistant form of p38α (DR), was determined the biological and physiological function of p38α in myocardial ischaemia [106]. The findings suggested that p38α is the major isoform of p38 MAPK contributing to myocardial ischaemia and support the cardioprotective effect of SB203580 [106], and also highlighted that myocardial ischaemia activates p38 MAPK by autophosphorylation.

CONCLUSION

Ischaemic heart disease remains, and is likely to continue to be, the leading life threatening disease around the world. Numerous studies from independent groups suggest that the p38-MAPK activation that accompanies myocardial ischaemia aggravates injury. However, under different circumstances activation of this kinase can reduce myocardial injury. The better understand the mechanisms of p38-MAPK activation during myocardial ischaemia is a likely prerequisite to the exploitation of the wealth of preclinical data that suggests inhibiting this kinase will benefit patients with ischemic heart disease. Using gene manipulation techniques and also transgenic animal models, many studies demonstrated the detrimental role of p38 MAPK activation. Inhibition of p38 activation by specific ATP competitive inhibitor, SB203580, is cardioprotection. However, these findings need further validate p38α as a viable clinical target and form the basis of future studies designed to further dissect the signaling pathways and discover the downstream substrates responsible for injury.

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