Published in final edited form as:

J Mol Cell Cardiol. 2011 October ; 51(4): 485–490. doi:10.1016/j.yjmcc.2010.10.021.

The p38 Mitogen-Activated Protein Kinase Pathway-A Potential Target for Intervention in Infarction, Hypertrophy and Heart Failure

Michael S. Marber¹, Beth Rose², and Yibin Wang²

¹ King's College London BHF Centre, Cardiovascular Division, The Rayne Institute, St. Thomas' Hospital, London SE1 7EH, United Kingdom

² Departments of Anesthesiology, Physiology and Medicine, UCLA, Los Angeles, CA 90095. USA

Abstract

The p38 mitogen-activated protein kinases (p38s) are stress activated ser/thr kinases. Their activation has been associated with various pathological stressors in the heart. Activated p38 is implicated in a wide spectrum of cardiac pathologies, including hypertrophy, myocardial infarction, as well as systolic and diastolic heart failure. In this review, the specific contribution of different isoforms of p38 kinases to cardiac diseases as well as TAB-1 mediated non-canonical activation pathway are discussed as a rationale for inhibiting p38 activity to treat cardiac hypertrophy, ischemic injury and heart failure. Finally, a summary of current clinical trials targeting p38 kinases in cardiovascular diseases is provided to highlight the potential promise as well as existing challenges of this therapeutic approach.

The p38 mitogen-activated protein kinases (p38s) are stress-activated kinases that together with the extracellular signal-regulated kinases 1/2 (ERK1/2), the c-Jun N-terminal kinases (JNKs) and extracellular signal-regulated kinase 5 (ERK5) form the 4 principal limbs of the mitogen-activated protein kinase (MAPKs) system. The MAPKs are serine/threonine protein kinases that can be activated by ligands of G-protein-coupled, and growth factor, receptors, as well as other stressors such as reactive oxidative species.

Isoforms of p38 and their mechanism of activation

Four p38 isoforms (α , β , γ and δ) exist which have preserved structure but variable sensitivity to pharmacological inhibition. p38 α and β have high sequence homology and share sensitivity to pharmacological inhibition by pyridinyl imidazole molecules (such as SB203580) but have only 60% homology with p38 γ and δ which are resistant to inhibition by SB203580[1].

Correspondence: Yibin Wang, Ph.D., Professor, Division of Molecular Medicine, Departments of Anesthesiology, Physiology and Medicine Molecular Biology Institute, David Geffen School of Medicine, UCLA. Room BH 569, CHS, 650 Charles E. Young Drive, Los Angeles, CA 90095. Tel: 310 206-5197. Fax: 310 206-5907. yibinwang@mednet.ucla.edu. Professor Michael Marber BSc, MB.BS, PhD, FRCP, Professor of Cardiology, King's College London, Honorary Consultant Cardiologist, Guy's & St Thomas' Hospitals, The Rayne Institute, St Thomas' Hospital, Lambeth Palace Rd, London SE1 7EH, Tel: +44-(0)20-7188 1008, Fax: +44-(0)20-7188 0970, mike.marber@kcl.ac.uk.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All investigators agree that p38 α exists in the myocardium of rodents, large animals and humans[2–9]. Similarly, amongst the few investigators that have performed a systematic examination, there is general agreement that p38 γ is preferentially expressed in cardiac muscle and is present at all stages of development[9–11]. Although several earlier studies also claimed p38 expression is rather restricted to skeletal muscle[12,13]. In contrast, it is generally accepted that p38 β and p38 δ are less abundant in the myocardium[2,10,14].

All four isoforms have a Thr-Gly-Tyr (TGY) dual phosphorylation motif/epitope which is used by investigators to infer activation. The traditional view is that this dual phosphorylation event is achieved by upstream, dual specificity, MAPK kinases (MAPKKs) or MKKs. The major activators of p38 *in vivo* are MKK3 and MKK6[15,16] with perhaps some contribution from MKK4[17,18]. 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, there is a misalignment of these lobes, that prevents the co-operation between Lys⁵³, in the N-terminal lobe, and Asp¹⁶⁸, in the C-terminal lobe, imperative to the binding and stabilisation of ATP[19,20]. The prediction therefore is that the non-dual phospho-form of p38-MAPK is inactive as a result of steric obstruction of the peptide-binding channel and low-ATP affinity. The MKK-induced, dual phosphorylation event is thought to cause the activation loop to refold[21] 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 the N-, and C-, terminal lobes so that Lys⁵³ and Asp¹⁶⁸ move towards one another by 2.5–5Å enabling the cooperation necessary for ATP binding[19] and restoring the active conformation of the “Catalytic Spine” of the kinase[22].

Thus based on structure and function, ATP binding occurs after, and not before, dual phosphorylation of the activation loop. Nonetheless, and of particular relevance to the heart, a number of lines of enquiry have suggested that p38s can also auto-activate through at least 2 distinct mechanisms, one involving a scaffold protein known as TGF- β -activated protein kinase 1 binding protein 1 (TAB1)[20,23] and the other a priming phosphorylation of Tyr323 by a tyrosine kinase of the SYK-related family[24,25].

The TAB1-facilitated autoactivation of myocardial p38 has now been described as a non-canonical mode of p38 activation by a number of independent groups and always seems to lead to adverse consequences[26–31]. This mechanism of activation, which is likely confined to p38 α , is worthy of further discussion since it may allow circumstance-selective inhibition of p38 activity, which cannot be achieved with the current agents in early phase clinical trial (see below).

TAB1 is a scaffold protein that promotes the autoactivation of another kinase, TGF- β -activated protein kinase 1 (TAK1), which is upstream of p38 and is capable of directly activating MKK3 and MKK6[32]. Furthermore TAB1 is a p38 substrate, suggesting it may also be a component of closed feedback loop regulating p38 activity[32,33]. This complexity obfuscates data interpretation. Nonetheless, TAB1 and p38 associate during myocardial ischaemia[26–28] and this association alone is capable of causing p38 activation[29,30,34]. Furthermore, a natural splice variant of TAB1 exists that can perform this function without interacting with TAK1[35]. Finally, the characteristic readout of autoactivation, a reduction in p38 dual phosphorylation when the ATP-binding site is occupied by a catalytic site inhibitor, is commonly observed when p38 is activated by cardiac stresses such as ischaemia (for some examples see figure 2 of Maulik et al[36], figure 4 of Clanachan et al[37], figure 2 of Yada et al[38], figure 3 of Aleshin et al[39], figure 10 of Capano et al[40], figure 4 of Gorog et al[41], figure 3 of Wang et al[42], figure 5 of House et al[43], figure 5A of Lau et al[44] and figure 4 of Sy et al[45]). The reason why

this is a readout of autoactivation is that p38 inhibitors do not interfere with the catalytic activity of MKK3/6 and thus would not interfere with transphosphorylation by an activating upstream kinase. Interestingly, Ota et al recently reported that Hsp90/Cdc37 also plays an inhibitory role in p38 autophosphorylation and selectively modulates TAB1 mediated p38 activation[30].

Isoforms of p38 and their pathophysiological role

Most of the studies to date have used pharmacological inhibition of p38 to infer its role in myocardial biology. The vast majority of these compounds only inhibit p38 α and p38 β and do so at very similar IC₅₀s. The use of these pharmacological agents has spawned a large literature that suggests the sustained activation of p38 contributes to myocyte death during lethal myocardial ischaemia and to cardiac dysfunction and fibrosis during post-infarction remodeling, hypertrophy and with transgenic expression of upstream activating kinases (see[33,46,47] for reviews). These studies have been reviewed elsewhere and will not be discussed further here, instead we will concentrate on possible isoform-specific functions. Using adenoviral-mediated expression of p38 α and β in rat neonatal cardiomyocytes, Saurin *et al* have previously shown that during simulated ischaemia p38 is activated, whereas p38 β is inhibited[4]. Inhibition during lethal ischaemia of p38 α , but not β , resulted in increased cell viability[4]. This strongly supports Wang *et al* who previously suggested that p38 α activation in cardiac myocytes causes apoptosis whereas activation of the isoform leads to protection and hypertrophy[3]. The possible protective role of p38 β has subsequently been highlighted by a number of investigators[5,8,47–49]. However, none of these studies involve manipulation of endogenous p38 β . Nonetheless, it has been suggested that ischaemic preconditioning is the result of selective activation of p38 β [4,5]. This perhaps explains why pharmacological inhibition of p38s α and β during preconditioning blocks protection, (since β is perhaps the dominant isoform activated), whilst during lethal ischaemia the same inhibitors, at the same concentrations, performed by the same investigators, often within the same study, cause protection, (since α is perhaps the dominant isoform activated)[26,50–53]. The detrimental effect of p38 α activation during ischaemia is also supported by studies that do not rely on pharmacological inhibition or ectopic overexpression of a wild-type isoform. For example, we have shown that the protective effect of SB203580 in isolated cardiomyocytes exposed to simulated ischaemia is lost in myocytes expressing a form of p38 α resistant to pharmacological inhibition[54]. Similarly, mice heterozygous for a p38 α null allele, with consequently reduced levels of myocardial p38 α protein, are resistant to infarction[55] as are mice transgenic for a kinase dead form of p38 α [56]. Thus there is strong direct evidence that the activation of p38 α during lethal ischaemia increases injury, whilst the activation of p38 β , most usually before ischaemia, maybe protective. Very recently, this paradigm has been challenged using knock-in mice where either the endogenous p38 α or p38 β alleles have been replaced with a gene encoding an identical isoform with a single residue substitution that renders it resistant to pharmacological inhibition. These studies confirm that sustained p38 α activation during lethal ischaemia does indeed aggravate injury[57] but that surprisingly brief activation of the same isoform during ischaemic preconditioning contributes to protection[58]. Furthermore, p38 β null mice could be preconditioned by transient ischaemia and there was no change in sensitivity to lethal ischaemia[58]. Similar results also suggest p38 α activity is required to adapt to hypertrophic stress. Global gene targeting to disrupt both p38 α alleles results in embryonic lethality[59,60] however myocyte-specific knockouts are viable but respond poorly to haemodynamic stress[61] again suggesting that under some circumstances even the activation of p38 α has beneficial consequences. We (Rose et al, unpublished results) have recently observed that cardiomyocyte specific knockout of both p38 α and p38 β in mice led to spontaneous right ventricular hypertrophy, suggesting that both isoforms may contribute to maintain normal development and function of the heart. In contrast to the chronic absence

of p38, acute activation results in hypertrophy and heart failure, in part mediated by the downstream kinase mitogen-activated protein kinase 2 (MAPKAPK2 or MK2)[62]. In summary, the physiological role, if any, of myocardial p38 β remains uncertain. In contrast there is compelling evidence that the activation of p38 α can aggravate cardiac injury, but tantalizingly there are also data to suggest it is involved in chronic adaptation to ischaemia[57] and to abrupt increases in after load[61].

In distinction to p38s α and β , there is little information in the literature regarding the roles of either or isoforms during myocardial ischaemia or hypertrophy. Since these isoforms lack a crucial “gatekeeper” residue (Thr106) within the ATP-binding pocket they are resistant to classic rationally designed ATP-competitive inhibitors. However, conserved cardiac expression of p38 and perhaps δ amongst several different species suggests that these isoforms play an important role in the heart and are unlikely to be functionally redundant[6,9,11,63,64]. This notion is further reinforced by the findings that their in vitro substrate preferences[64–68] and scaffold partners[69] differ from those of α and β . Although p38 was reported to be highly expressed in heart and translocated from cytoplasm to nuclei upon pressure-overload, its specific role in the heart is still unknown[10]. In short, although the relative contribution of individual p38 isoforms to cardiac pathologies still needs to be further defined, based on current evidence p38 α appears to be the major player.

Despite an extensive body of literature describing the phenotypic consequences of p38 manipulation, surprisingly little is known about their underlying molecular basis. Myocyte death, pro-inflammatory gene induction, myofilament modulation and extracellular matrix remodeling are among the main factors contributing to the deleterious effects of p38 activation. The beneficial effects of activation include an anti-hypertrophic action and enhanced myocardial adaptation to stress perhaps through myocardial angiogenesis. However, the p38 substrates and interacting partners that mediate these effects remain unknown and in urgent need of study.

Pharmacological inhibition of p38

As mentioned above, and reviewed elsewhere, there is compelling evidence that pharmacological inhibition of p38 enhances contractile performance, reduces ischaemic injury and consequently heart failure in animal models. Furthermore, it seems likely similar mechanisms could operate in the human heart since myocardial p38 is identically activated in patients with ischaemia[70,71] and/or heart failure[72,73]. It would therefore seem reasonable to attempt to inhibit p38 as a therapeutic strategy. Fairly specific inhibitors of p38 have existed for almost 2 decades and in fact their anti-inflammatory activity led to the discovery of p38 α as a proinflammatory kinase[74]. As a consequence, the structure-activity relationship of p38 with an archetypal inhibitor was described more than a decade ago[20]. Given the necessary conservation of key aspects of the ATP-binding pocket amongst the kinome it is inevitable ATP competitive inhibitors lack selectivity. Nonetheless, it is acknowledged that such inhibitors targeting p38 are often more selective than many ATP-competitive inhibitors developed for other kinases[75]. Over the years a number of other structural features of p38 have been discovered that can be exploited by medicinal chemists to create ever more potent and selective inhibitors. Some of these features, such as the Asp-Phe-Gly (DFG)-motif sequence at the base of the activation loop, are common to all 4 isoforms enabling pharmacological inhibition of p38 γ and p38 δ [76,77]. As summarized in a recent excellent review,[78] the 3 principal features that have been successfully exploited in designing inhibitors are (i) DFG binding, largely to increase potency since this motif is highly conserved amongst kinases; (ii) the small gatekeeper Thr residue (106 in p38 α and p38 β , equivalent residue in p38 γ and p38 δ is Met), which also confers selectivity since it is

found in less than 20% of kinases and (iii) a mobile Gly110 adjacent to the linker Met109, which also confers selectivity occurring in less than 10% of kinases.

A large number of scaffolds that inhibit p38 have been patented however only a small number have proceeded to clinical trial and of these none have progressed beyond phase 2. The principal reasons for this lack of progress are toxicity which has limited the doses at which inhibitors can be used. In the most recent trials there has also been limited anti-inflammatory efficacy perhaps caused by the use of relatively low doses, p38 escape (see below) and/or potent anti-inflammatory background therapy.

Clinical trials with p38 inhibitors

To date the majority of the experience with p38 inhibition has been for inflammatory, non-cardiac, conditions. Information from such trials has recently entered the public domain. The conditions that have been reported on most extensively are rheumatoid arthritis and Crohn's disease. Since these are not directly relevant to the cardiovascular system interested readers are directed to recent reviews[79,80]. The "take home" message from these trials is that despite some favourable trends inhibition of p38 has not shown efficacy when added to traditional anti-inflammatory disease modifying drugs and there have been signs of toxicity most notably with an increase in intercurrent infections, raised liver enzymes and skin rashes. One very interesting observation common to a number of trials is escape from p38 inhibition with chronic therapy that is discussed in more detail below under unresolved issues requiring future research.

Of more interest to the readership of this journal are trials of p38 inhibition for cardiovascular disease. Thus far these have been limited to small detailed pathophysiological studies only published as preliminary abstracts. Vertex's compound VX-702 was used in a dose-escalation study in 45 patients undergoing planned coronary artery angioplasty (PCI)[81]. The salient finding was a suppression of the elevation in C reactive protein (CRP) and other inflammatory markers (monocytes, neutrophils and total white blood cell count) for 3–4 days post procedure. Oral dosing with the inhibitor (5–40 mg, once per day) was for 5 days starting the day before the procedure and was well tolerated[81]. In a study of a similar design SB681323 at a dose of 7.5 mg per day was compared to placebo in 92 patients with 1:1 randomisation (ClinicalTrials.gov identifier NCT00291902). Dosing in this double blind study was for 28 days starting 3 days before planned PCI. SB681323 was associated with a reduction in CRP at day 5 and day 28 post procedure[82,83]. There were also reductions in other inflammatory markers such as myeloperoxidase and IL8. The study was not powered to look at clinical endpoints but there were a significant reductions in episodes of angina and the need for further revascularisation, albeit on a post-hoc analysis[82,83].

Based on ClinicalTrials.gov both GlaxoSmithKline (with GW85655 also known as Losmapimod, identifier NCT00633022) and Bristol-Myers Squibb (with BMS582949, identifier NCT00570752) have active programs examining the effects of p38 inhibition on inflammation within atherosclerotic plaques as assessed by ¹⁸F-Fluorodeoxyglucose (FDG) uptake on PET/CT. The GSK study with over 100 patients has completed recently but has not reported. In this study 2 dosing regimens of Losmapimod at 7.5 mg o.d. or 7.5 mg b.i.d. were compared with placebo. The BMS study is ongoing with a target of 70 patients dosed with BMS582949 at 100 mg o.d. against placebo. Completion is expected in Jan 2011. In both studies dosing is for 3 months with a presumed primary endpoint of change in FDG uptake at baseline compared with 12 weeks. The study design and the endpoints are based on preclinical translational studies demonstrating that p38 activity within macrophages contributes to atherosclerosis progression[84].

Perhaps the most interesting and ambitious study is in the setting of non-ST Elevation Myocardial Infarction (NSTEMI) by GlaxoSmithKline (with Losmapimod at 7.5 mg b.i.d. with or without a 15 mg loading dose for 12 weeks compared to placebo, see ClinicalTrials.gov NCT00910962). In this study 500 pts with NSTEMI will be randomized. The primary endpoints relate principally to safety in this high risk population. However, there are some measures of efficacy such as alterations in CRP and measures of infarct size based on biomarkers. In addition there is a substudy in which changes in infarction and cardiac function will be analyzed by MRI. The study is due to complete in early 2011.

Alternative Strategies to Target the p38 Pathway for Heart Disease

The lessons learnt from current p38 inhibitors for inflammatory arthritis and inflammatory bowel disease suggest that preclinical data cannot necessarily predict clinical response. There appear to be two main challenges facing the current p38 kinase targeted therapies, one is escape of efficacy from chronic inhibition and another is potential side-effects in unintended organs.

With chronic studies in man it is clear that p38 escapes from inhibition since the initial suppression of systemic cytokine concentrations is transient. The reasons why p38 inhibition only results in transient suppression of inflammation are complex but they likely relate to activation of the feedback loops known to control activity. A variety of mechanisms that allow p38 to escape inhibition have been reviewed recently[85] and include the feedback mechanism mentioned above involving the protein TAB1. In addition, p38 kinases may function as a major signaling conduit of compensatory stress response, thus systemic inhibition may lead to undesired consequences. Therefore, finding an alternative strategy to target the p38 pathway would be valuable. Based on our current understanding of p38 regulation, targeting the TAB1-mediated non-canonical activation of p38 has a number of attractions. This non-canonical mode of p38 activation appears to occur relatively specifically in certain circumstances, such as ischemic hearts and differs from the canonical activation of p38 by upstream kinases that leads to inflammation[29]. Furthermore, it appears that the TAB1-mediated non-canonical p38 pathway can be regulated by a different set of molecular partners from the canonical pathway, such as Hsp90/Cdc37. It therefore has the possible attraction of circumstance specificity and potentially would avoid the escape mechanisms occurring through pathways that are amplified with inhibitors of p38 catalytic activity. It would also potentially avoid the toxicity seen with classic inhibitors that likely relates to the key homeostatic function of p38 α [59,86]. To achieve these goals, we would need to have a better understanding at the molecular level of the different regulatory mechanisms controlling p38 under specific pathological conditions. In this case, uncovering the structural basis for the p38 and TAB-1 interaction and its regulation by other molecular partners would be an essential step leading to more effective p38 targeted therapies for ischemic heart diseases. As an example we have recently described a mechanism responsible for regulating the interaction between TAB1 and p38 α in the heart that if potentiated could prevent activation during myocardial ischaemia[30]. What is certain is that a more complete understanding of the mechanisms of p38 activation and of the relevant downstream substrates will significantly advance the field and enable more refined therapies targeting this crucial kinase.

Reference List

1. Eyers PA, van den Ijssel P, Quinlan RA, Goedert M, Cohen P. Use of a drug-resistant mutant of stress-activated protein kinase 2 α /p38 to validate the in vivo specificity of SB203580. *FEBS Letts.* 1999; 451:191–6. [PubMed: 10371163]

2. Rakhit RD, Kabir AN, Mockridge JW, Saurin A, Marber MS. Role of G proteins and modulation of p38 MAPK activation in the protection by nitric oxide against ischemia-reoxygenation injury. *Biochem Biophys Res Commun.* 2001; 286:995–1002. [PubMed: 11527399]
3. Wang Y, Huang S, Sah VP, Ross J, Brown JH, Han J, et al. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen activated protein kinase family. *J Biol Chem.* 1998; 273(4):2161–8. [PubMed: 9442057]
4. Saurin AT, Martin JL, Heads RJ, Foley C, Mockridge JW, Wright MJ, et al. The role of differential activation of p38-mitogen-activated protein kinase in preconditioned ventricular myocytes. *FASEB J.* 2000; 14:2237–46. [PubMed: 11053245]
5. Schulz R, Gres P, Skyschally A, Duschin A, Belosjorow S, Konietzka I, et al. Ischemic preconditioning preserves connexin 43 phosphorylation during sustained ischemia in pig hearts in vivo. *Faseb J.* 2003; 17:1355–7. [PubMed: 12759340]
6. Lemke LE, Bloem LJ, Fouts R, Esterman M, Sandusky G, Vlahos CJ. Decreased p38 MAPK activity in end-stage failing human myocardium: p38 MAPK alpha is the predominant isoform expressed in human heart. *J Mol Cell Cardiol.* 2001; 33:1527–40. [PubMed: 11448140]
7. Vahebi S, Ota A, Li M, Warren CM, de Tombe PP, Wang Y, et al. p38-MAPK induced dephosphorylation of alpha-tropomyosin is associated with depression of myocardial sarcomeric tension and ATPase activity. *Circ Res.* 2007; 100:408–15. [PubMed: 17234967]
8. Martindale JJ, Wall JA, Martinez-Longoria DM, Aryal P, Rockman HA, Guo Y, et al. Overexpression of Mitogen-activated Protein Kinase Kinase 6 in the Heart Improves Functional Recovery from Ischemia in Vitro and Protects against Myocardial Infarction in Vivo. *J Biol Chem.* 2005; 280:669–76. [PubMed: 15492008]
9. Beardmore VA, Hinton HJ, Eftychi C, Apostolaki M, Armaka M, Darragh J, et al. Generation and characterization of p38beta (MAPK11) gene-targeted mice. *Mol Cell Biol.* 2005; 25:10454–64. [PubMed: 16287858]
10. Dingar D, Merlen C, Grandy S, Gillis MA, Villeneuve LR, Mamarbachi AM, et al. Effect of pressure overload-induced hypertrophy on the expression and localization of p38 MAP kinase isoforms in the mouse heart. *Cell Signal.* 2010; 22:1634–44. [PubMed: 20600854]
11. Court NW, dos Remedios CG, Cordell J, Bogoyevitch MA. Cardiac Expression and Subcellular Localization of the p38 Mitogen-activated Protein Kinase Member, Stress-activated Protein Kinase-3 (SAPK3). *J Mol Cell Cardiol.* 2002; 34:413–26. [PubMed: 11991731]
12. Li Z, Jiang Y, Ulevitch RJ, Han J. The primary structure of p38 gamma: a new member of p38 group of MAP kinases. *Biochem Biophys Res Commun.* 1996; 228:334–40. [PubMed: 8920915]
13. Lechner C, Zahalka MA, Giot JF, Moller NP, Ullrich A. ERK6, a mitogen-activated protein kinase involved in C2C12 myoblast differentiation. *Proc Natl Acad Sci U S A.* 1996; 93:4355–9. [PubMed: 8633070]
14. Liao P, Georgakopoulos D, Kovacs A, Zheng MZ, Lerner D, Pu HY, et al. The in vivo role of p38 MAP kinases in cardiac remodeling and restrictive cardiomyopathy. *Proc Natl Acad Sci U S A.* 2001; 98:12283–8. [PubMed: 11593045]
15. Raingeaud J, Whitmarsh AJ, Barrett T, Derijard B, Davis RJ. MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol Cell Biol.* 1996; 16:1247–55. [PubMed: 8622669]
16. Derijard B, Raingeaud J, Barrett T, Wu IH, Han J, Ulevitch RJ, et al. Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science.* 1995; 267:682–5. [PubMed: 7839144]
17. Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kelkar N, Tanaka Y, et al. Mechanism of p38 MAP kinase activation in vivo. *Genes Dev.* 2003; 17:1969–78. [PubMed: 12893778]
18. Kang YJ, Seit-Nebi A, Davis RJ, Han J. Multiple activation mechanisms of p38alpha mitogen-activated protein kinase. *J Biol Chem.* 2006; 281:26225–34. [PubMed: 16849316]
19. Wilson KP, Fitzgibbon MJ, Caron PN, Griffith JP, Chen WY, McCaffrey PG, et al. Crystal structure of p38 mitogen-activated protein kinase. *J Biol Chem.* 1996; 271:27696–700. [PubMed: 8910361]
20. Gum RJ, McLaughlin MM, Kumar S, Wang ZL, Bower MJ, Lee JC, et al. Acquisition of sensitivity of stress-activated protein kinases to the p38 inhibitor, SB 203580, by alteration of one

- or more amino acids within the ATP binding pocket. *J Biol Chem.* 1998; 273:15605–10. [PubMed: 9624152]
21. Canagarajah BJ, Khokhlatchev A, Cobb MH, Goldsmith EJ. Activation mechanism of the MAP kinase ERK2 by dual phosphorylation. *Cell.* 1997; 90:859–69. [PubMed: 9298898]
 22. Kornev AP, Taylor SS, Ten Eyck LF. A helix scaffold for the assembly of active protein kinases. *Proc Natl Acad Sci U S A.* 2008; 105:14377–82. [PubMed: 18787129]
 23. Johnson G. Signal transduction - Scaffolding proteins - More than meets the eye. *Science.* 2002; 295:1249–50. [PubMed: 11847330]
 24. Salvador JM, Mittelstadt PR, Guszczynski T, Copeland TD, Yamaguchi H, Appella E, et al. Alternative p38 activation pathway mediated by T cell receptor-proximal tyrosine kinases. *Nat Immunol.* 2005; 6:390–5. [PubMed: 15735648]
 25. Mocsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat Rev Immunol.* 2010; 10:387–402. [PubMed: 20467426]
 26. Tanno M, Bassi R, Gorog DA, Saurin AT, Jiang J, Heads RJ, et al. Diverse mechanisms of myocardial p38 mitogen-activated protein kinase activation: evidence for MKK-independent activation by a TAB1-associated mechanism contributing to injury during myocardial ischemia. *Circ Res.* 2003; 93:254–61. [PubMed: 12829618]
 27. Li J, Miller EJ, Ninomiya-Tsuji J, Russell RR III, Young LH. AMP-Activated Protein Kinase Activates p38 Mitogen-Activated Protein Kinase by Increasing Its Recruitment to TAB1 in the Ischemic Heart. *Circ Res.* 2005; 97:872–9. [PubMed: 16179588]
 28. Fiedler B, Feil R, Hofmann F, Willenbockel C, Drexler H, Smolenski A, et al. cGMP-dependent protein kinase type I inhibits TAB1-p38 mitogen-activated protein kinase apoptosis signaling in cardiac myocytes. *J Biol Chem.* 2006; 281:32831–40. [PubMed: 16943189]
 29. Lu G, Kang YJ, Han J, Herschman HR, Stefani E, Wang Y. TAB-1 modulates intracellular localization of p38 MAP kinase and downstream signaling. *J Biol Chem.* 2006; 281:6087–95. [PubMed: 16407200]
 30. Ota A, Zhang J, Ping P, Han J, Wang Y. Specific Regulation of Noncanonical p38{alpha} Activation by Hsp90-Cdc37 Chaperone Complex in Cardiomyocyte. *Circ Res.* 2010; 106:1404–12. [PubMed: 20299663]
 31. Shi J, Guan J, Jiang B, Brenner DA, Del MF, Ward JE, et al. Amyloidogenic light chains induce cardiomyocyte contractile dysfunction and apoptosis via a non-canonical p38alpha MAPK pathway. *Proc Natl Acad Sci U S A.* 2010; 107:4188–93. [PubMed: 20150510]
 32. Cheung PC, Campbell DG, Nebreda AR, Cohen P. Feedback control of the protein kinase TAK1 by SAPK2a/p38alpha. *EMBO J.* 2003; 22:5793–805. [PubMed: 14592977]
 33. Clark JE, Sarafraz N, Marber MS. Potential of p38-MAPK inhibitors in the treatment of ischaemic heart disease. *Pharmacol Ther.* 2007; 116:192–206. [PubMed: 17765316]
 34. Ge BX, Gram H, Di Padova F, Huang B, New L, Ulevitch RJ, et al. MAPKK-independent activation of p38 alpha mediated by TAB1-dependent autophosphorylation of p38 alpha. *Science.* 2002; 295:1291–4. [PubMed: 11847341]
 35. Ge B, Xiong X, Jing Q, Mosley JL, Filose A, Bian D, et al. TAB1beta (transforming growth factor-beta-activated protein kinase 1-binding protein 1beta), a novel splicing variant of TAB1 that interacts with p38alpha but not TAK1. *J Biol Chem.* 2003; 278:2286–93. [PubMed: 12429732]
 36. Maulik N, Yoshida T, Zu Y-L, Sat M, Banerjee A, Das DK. Ischemic preconditioning triggers tyrosine kinase signalling: A potential role for MAPKAP kinase 2. *Am J Physiol.* 1998; 275:H1857–H1864. [PubMed: 9815094]
 37. Clanachan AS, Jaswal JS, Gandhi M, Bottorff DA, Coughlin J, Finegan BA, et al. Effects of inhibition of myocardial extracellular-responsive kinase and P38 mitogen-activated protein kinase on mechanical function of rat hearts after prolonged hypothermic ischemia. *Transplantation.* 2003; 75:173–80. [PubMed: 12548118]
 38. Yada M, Shimamoto A, Hampton CR, Chong AJ, Takayama H, Rothnie CL, et al. FR167653 diminishes infarct size in a murine model of myocardial ischemia-reperfusion injury. *J Thorac Cardiovasc Surg.* 2004; 128:588–94. [PubMed: 15457160]

39. Aleshin A, Sawa Y, Ono M, Funatsu T, Miyagawa S, Matsuda H. Myocardial protective effect of FR167653; a novel cytokine inhibitor in ischemic-reperfused rat heart. *European Journal of Cardio-Thoracic Surgery*. 2004; 26:974–80. [PubMed: 15519192]
40. Capano M, Crompton M. Bax translocates to mitochondria of heart cells during simulated ischaemia: involvement of AMP-activated and p38 mitogen-activated protein kinases. *Biochem J*. 2006; 395:57–64. [PubMed: 16321138]
41. Gorog DA, Tanno M, Cao X, Bellahcene M, Bassi R, Kabir AM, et al. Inhibition of p38 MAPK activity fails to attenuate contractile dysfunction in a mouse model of low-flow ischemia. *Cardiovasc Res*. 2004; 61:123–31. [PubMed: 14732209]
42. Wang M, Tsai BM, Turrentine MW, Mahomed Y, Brown JW, Meldrum DR. p38 mitogen activated protein kinase mediates both death signaling and functional depression in the heart. *Ann Thorac Surg*. 2005; 80:2235–41. [PubMed: 16305880]
43. House SL, Branch K, Newman G, Doetschman T, Schultz JJ. Cardioprotection induced by cardiac-specific overexpression of fibroblast growth factor-2 is mediated by the MAPK cascade. *Am J Physiol Heart Circ Physiol*. 2005; 289:H2167–H2175. [PubMed: 16040717]
44. Lau JM, Jin X, Ren J, Avery J, Debosch BJ, Treskov I, et al. The 14–3–3{tau} Phosphoserine-Binding Protein Is Required for Cardiomyocyte Survival. *Mol Cell Biol*. 2007; 27:1455–66. [PubMed: 17145769]
45. Sy JC, Seshadri G, Yang SC, Brown M, Oh T, Dikalov S, et al. Sustained release of a p38 inhibitor from non-inflammatory microspheres inhibits cardiac dysfunction. *Nat Mater*. 2008; 7:863–8. [PubMed: 18931671]
46. Kerkela R, Force T. p38 mitogen-activated protein kinase: a future target for heart failure therapy? *J Am Coll Cardiol*. 2006; 48:556–8. [PubMed: 16875983]
47. Wang Y. Mitogen-activated protein kinases in heart development and diseases. *Circulation*. 2007; 116:1413–23. [PubMed: 17875982]
48. Kim HP, Wang X, Zhang J, Suh GY, Benjamin IJ, Ryter SW, et al. Heat shock protein-70 mediates the cytoprotective effect of carbon monoxide: involvement of p38 beta MAPK and heat shock factor-1. *J Immunol*. 2005; 175:2622–9. [PubMed: 16081837]
49. Kim JK, Pedram A, Razandi M, Levin ER. Estrogen Prevents Cardiomyocyte Apoptosis through Inhibition of Reactive Oxygen Species and Differential Regulation of p38 Kinase Isoforms. *J Biol Chem*. 2006; 281:6760–7. [PubMed: 16407188]
50. Sanada S, Kitakaze M, Papst PJ, Hatanaka K, Asanuma H, Aki T, et al. Role of phasic dynamism of p38 mitogen-activated protein kinase activation in ischemic preconditioning of the canine heart. *Circulation Research*. 88:175–80. [PubMed: 11157669]
51. Nagarkatti DS, Ramadan IS. Role of p38 MAP kinase in myocardial stress. *J Mol Cell Cardiol*. 1998; 30:1651–64. [PubMed: 9841266]
52. Marais E, Genade S, Huisamen B, Strijdom JG, Moolman JA, Lochner A. Activation of p38 MAPK induced by a multi-cycle ischaemic preconditioning protocol is associated with attenuated p38 MAPK activity during sustained ischaemia and reperfusion. *J Mol Cell Cardiol*. 2001; 33:769–78. [PubMed: 11273729]
53. Sanada S, Kitakaze M, Papst PJ, Hatanaka K, Asanuma H, Aki T, et al. Role of phasic dynamism of p38 mitogen-activated protein kinase activation in ischemic preconditioning of the canine heart. *Circulation Research*. 2001; 88:175–80. [PubMed: 11157669]
54. Martin JL, Avkiran M, Quinlan RA, Cohen P, Marber MS. Antiischemic effects of SB203580 are mediated through the inhibition of p38alpha mitogen-activated protein kinase: Evidence from ectopic expression of an inhibition-resistant kinase. *Circ Res*. 2001; 89:750–2. [PubMed: 11679403]
55. Otsu K, Yamashita N, Nishida K, Hirotani S, Yamaguchi O, Watanabe T, et al. Disruption of a single copy of the p38alpha MAP kinase gene leads to cardioprotection against ischemia-reperfusion. *Biochem Biophys Res Commun*. 2003; 302:56–60. [PubMed: 12593847]
56. Kaiser RA, Bueno OF, Lips DJ, Doevendans PA, Jones F, Kimball TF, et al. Targeted inhibition of p38 mitogen-activated protein kinase antagonizes cardiac injury and cell death following ischemia-reperfusion in vivo. *J Biol Chem*. 2004; 279:15524–30. [PubMed: 14749328]

57. Kumphune S, Bassi R, Jacquet S, Sicard P, Clark JE, Verma S, et al. A chemical genetic approach reveals that p38alpha MAPK activation by diphosphorylation aggravates myocardial infarction and is prevented by the direct binding of SB203580. *J Biol Chem.* 2010; 285:2968–75. [PubMed: 19996096]
58. Sicard P, Clark JE, Jacquet S, Mohammadi S, Arthur JS, O'Keefe SJ, et al. The activation of p38alpha, and not p38beta, mitogen-activated protein kinase is required for ischemic preconditioning. *J Mol Cell Cardiol.* 2010; 48:1324–8. [PubMed: 20188737]
59. Mudgett JS, Ding J, Guh-Siesel L, Chartrain NA, Yang L, Gopal S, et al. Essential role for p38alpha mitogen-activated protein kinase in placental angiogenesis. *Proc Natl Acad Sci U S A.* 2000; 97:10454–9. [PubMed: 10973481]
60. Adams RH, Porras A, Alonso G, Jones M, Vintersten K, Panelli S, et al. Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development. *Mol Cell.* 2000; 6:109–16. [PubMed: 10949032]
61. Nishida K, Yamaguchi O, Hirotani S, Hikoso S, Higuchi Y, Watanabe T, et al. p38alpha mitogen-activated protein kinase plays a critical role in cardiomyocyte survival but not in cardiac hypertrophic growth in response to pressure overload. *Mol Cell Biol.* 2004; 24:10611–20. [PubMed: 15572667]
62. Streicher JM, Ren S, Herschman H, Wang Y. MAPK-activated protein kinase-2 in cardiac hypertrophy and cyclooxygenase-2 regulation in heart. *Circ Res.* 2010; 106:1434–43. [PubMed: 20339119]
63. Mertens S, Craxton M, Goedert M. SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases. *FEBS Lett.* 1996; 383:273–6. [PubMed: 8925912]
64. Wang H, Xu Q, Xiao F, Jiang Y, Wu Z. Involvement of the p38 Mitogen-activated Protein Kinase {alpha}, {beta}, and {gamma} Isoforms in Myogenic Differentiation. *Mol Biol Cell.* 2008
65. Cuenda A, Rousseau S. p38 MAP-Kinases pathway regulation, function and role in human diseases. *Biochim Biophys Acta.* 2007
66. Sabio G, Arthur JS, Kuma Y, Pegg M, Carr J, Murray-Tait V, et al. p38gamma regulates the localisation of SAP97 in the cytoskeleton by modulating its interaction with GKAP. *EMBO J.* 2005; 24:1134–45. [PubMed: 15729360]
67. Hasegawa M, Cuenda A, Spillantini MG, Thomas GM, Buee-Scherrer V, Cohen P, et al. Stress-activated protein kinase-3 interacts with the PDZ domain of alpha1-syntrophin. A mechanism for specific substrate recognition. *J Biol Chem.* 1999; 274:12626–31. [PubMed: 10212242]
68. Kuma Y, Campbell DG, Cuenda A. Identification of glycogen synthase as a new substrate for stress-activated protein kinase 2b/p38beta. *Biochem J.* 2004; 379:133–9. [PubMed: 14680475]
69. Kelkar N, Standen CL, Davis RJ. Role of the JIP4 scaffold protein in the regulation of mitogen-activated protein kinase signaling pathways. *Mol Cell Biol.* 2005; 25:2733–43. [PubMed: 15767678]
70. Corbucci GG, Perrino C, Donato G, Ricchi A, Lettieri B, Troncone G, et al. Transient and reversible deoxyribonucleic acid damage in human left ventricle under controlled ischemia and reperfusion. *J Am Coll Cardiol.* 2004; 43:1992–9. [PubMed: 15172403]
71. Cook SA, Sugden PH, Clerk A. Activation of c-jun N-terminal kinases and p38-mitogen-activated protein kinases in human heart failure secondary to ischaemic heart disease. *J Mol Cell Cardiol.* 1999; 31:1429–34. [PubMed: 10423341]
72. Talmor D, Applebaum A, Rudich A, Shapira Y, Tirosh A. Activation of mitogen-activated protein kinases in human heart during cardiopulmonary bypass. *Circ Res.* 2000; 86:1004–7. [PubMed: 10807874]
73. Nediani C, Borch E, Giordano C, Baruzzo S, Ponziani V, Sebastiani M, et al. NADPH oxidase-dependent redox signaling in human heart failure: Relationship between the left and right ventricle. *J Mol Cell Cardiol.* 2007
74. Lee JC, Laydon JY, McDonnell PC, Gallagher TF, Kumar S, Green D, et al. A protein-kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature.* 1994; 372:739–46. [PubMed: 7997261]
75. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, et al. The selectivity of protein kinase inhibitors: a further update. *Biochem J.* 2007; 408:297–315. [PubMed: 17850214]

76. Pargellis C, Tong L, Churchill L, Cirillo PF, Gilmore T, Graham AG, et al. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat Struct Biol.* 2002; 9:268–72. [PubMed: 11896401]
77. Kuma Y, Sabio G, Bain J, Shpiro N, Marquez R, Cuenda A. BIRB796 inhibits all p38 MAPK isoforms in vitro and in vivo. *J Biol Chem.* 2005; 280:19472–9. [PubMed: 15755732]
78. Karcher SC, Laufer SA. Successful structure-based design of recent p38 MAP kinase inhibitors. *Curr Top Med Chem.* 2009; 9:655–76. [PubMed: 19689372]
79. Hammaker D, Firestein GS. “Go upstream, young man”: lessons learned from the p38 saga. *Ann Rheum Dis.* 2010; 69 (Suppl 1):i77–i82. [PubMed: 19995751]
80. Goldstein DM, Kuglstatter A, Lou Y, Soth MJ. Selective p38alpha inhibitors clinically evaluated for the treatment of chronic inflammatory disorders. *J Med Chem.* 2010; 53:2345–53. [PubMed: 19950901]
81. de Winter, RJ. AHA Scientific Sessions ePosters Archive 2005 poster 606. 2005. A major determinant of CRP production in patients with ACS undergoing PCI. The p38-MAPK signaling pathway. Ref Type: Internet Communication
82. Sarov-blat L, Morgan J, Fernandez P, James R, LePore JJ, Fang Z, et al. Inhibition of p38MAPK reduces inflammatory burden in subjects undergoing elective PCI. *J Am Col Cardiol.* 2009; 53:A422. Ref Type: Abstract.
83. sarov-blat, l; Morgan, JM.; Fernandez, P.; James, R.; Fang, Z.; Hurle, MR., et al. Inhibition of p38 Mitogen-Activated Protein Kinase Reduces Inflammation After Coronary Vascular Injury in Humans. *Arterioscler Thromb Vasc Biol.* 2010
84. Morris JB, Olzinski AR, Bernard RE, Aravindhan K, Mirabile RC, Boyce R, et al. p38 MAPK inhibition reduces aortic ultrasmall superparamagnetic iron oxide uptake in a mouse model of atherosclerosis: MRI assessment. *Arterioscler Thromb Vasc Biol.* 2008; 28:265–71. [PubMed: 18162612]
85. Cohen P. Targeting protein kinases for the development of anti-inflammatory drugs. *Curr Opin Cell Biol.* 2009; 21:317–24. [PubMed: 19217767]
86. Tamura K, Sudo T, Senftleben U, Dadak AM, Johnson R, Karin M. Requirement for p38 alpha in erythropoietin expression: A role for stress kinases in erythropoiesis. *Cell.* 2000; 102:221–31. [PubMed: 10943842]

Table 1

Summary of cardiovascular trials of p38 inhibition in the public domain

Compound	Sponsor	Registration No (clinicaltrials.gov)	Clinical Circumstance	Endpoint/Results
VX-702	Vertex	Unknown	PCI/Short term	Reduced Inflammatory Markers
SB681323	GSK	00291902	PCI/Medium term	Reduced inflammatory markers
GW85655 (Losmapimod)	GSK	00633022	Active atherosclerosis	FDG uptake in great vessels. Due to report at AHA 2010
BMS582949	BMS	00570752	Active atherosclerosis	FDG uptake in great vessels. Due to complete early 2011
GW85655 (Losmapimod)	GSK	00910962	Non-ST elevation MI	Safety/Inflammation. Due to complete 2011