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Developing small molecules to inhibit kinases unkind to the heart: p38 MAPK as a case in point

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Abstract

Over the last 40 years targeting G protein-coupled receptors and their ligands has had a major impact on the treatment of cardiovascular disease. However, the last decade has seen little progress and focus has shifted, particularly in the field of cancer biology, to downstream kinases. This review focuses on the kinases within the heart that become active during myocardial infarction and heart failure and contribute to cardiac dysfunction, with a special emphasis on p38 mitogen-activated protein kinase (MAPK).

Introduction

Heart failure is a leading cause of morbidity and mortality and is extremely expensive to treat. The targeting of “proximal” G-protein coupled receptors has been the major focus of the pharmaceutical industry over the last 4 decades. Although this research has delivered drugs that improve heart failure mortality, outlook still remains dismal with up to 40% of newly diagnosed patients being hospitalized or dying within 12 months despite blockade of β - and α -adrenoceptors and the renin-angiotensin-aldosterone system. The targeting of “downstream” protein kinases has now become the primary focus of industry and is having a major impact in cancer treatment. The benefit has so far been restricted to oncology because it is the field in which the biological role of kinases was first investigated, is best understood and therefore rational translation of findings is possible. In this review we highlight those kinases that have been implicated in the aggravation of heart failure and illustrate the pathway to developing pharmacological inhibitors by focusing on the p38 mitogen-activated protein kinases.

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What causes heart failure?

As hypertension is treated more aggressively, the etiology underlying chronic heart failure has shifted from being primarily hypertensive to being primarily ischaemic. After myocardial infarction (MI) a series of events occur which ultimately lead to progressive left ventricular dilatation and heart failure. Surprisingly, the process of progressive dilatation of the left ventricle is most usually independent of further episodes of MI and may become autonomous through varied mechanisms. A simplified framework to describe the complex pathophysiology is to divide the process into a stage of acute myocardial death as a result of infarction, expansion of the area of infarction as a result of poor healing and secondary changes in the remote myocardium away from the area of infarction that lead to hypertrophy, myocyte death and fibrosis. A multitude of kinases are activated during each of these processes and their inhibition by pharmacological or genetic means has been shown to benefit one or more stages of the pathophysiological process leading to heart failure (See table 1 for review). Amongst this plethora of kinases implicated in the pathophysiology of heart disease are the p38 mitogen-activated protein kinases (p38s).^{1–3}

Structural determinants of p38 utilized by inhibitors

The p38 family comprises 4 isoforms α , β , γ and δ of which α and γ are most highly expressed within the heart. The α and β isoforms share a gatekeeper Threonine residue at position 106 (Thr 106) in both human and murine isoforms.⁴ This gatekeeper guards a hydrophobic (back)pocket(I) probed by the classic pyridinylimidazole inhibitors discovered to have anti-inflammatory activity through inhibition of this pathway almost two decades ago.⁵ The terms in parenthesis apply to the nomenclature adopted to describe the 5 sub-regions of ATP-binding site used in the design of ATP-competitive inhibitors.⁵ A number of other scaffolds that also rely on this gatekeeper have also been developed (see^{5–8} for reviews). In common with the pyridinylimidazoles these molecules do not avidly bind the ATP pocket of the γ and δ isoforms which have a bulkier methionine as the gatekeeper at the equivalent position. The gatekeeper residue is crucial to the selectivity of the ATP-competitive inhibitors⁹ and kinases such as ERK2, p38 γ and p38 δ exhibit a 4 to 5-fold order of magnitude reduction in the IC₅₀ of pyridinylimidazoles when their gatekeepers are mutated to Thr.

The non-ATP competitive inhibitors of p38 bind in addition to the Asp-Phe-Gly (DFG)-motif at the base of the activation loop which is common to all isoforms.^{5,10} Such binding exerts an allosteric effect such that the kinase adopts and maintains a conformation that is resistant to activation by dual phosphorylation.¹¹ Consequently the allosteric inhibitors such as BIRB796 are able to inhibit all four p38 isoforms. However, since binding only occurs when p38s adopt a relatively rare conformation,¹¹ the IC₅₀s and on/off rates between isoforms vary, enabling differential inhibition.¹² The use and optimization of these two principal types of inhibitors are further discussed below. In addition to these direct p38 inhibitors there exist a range of other molecules that inhibit p38s indirectly by interacting with and modulating upstream pathways often through ill-defined mechanisms. These have been reviewed elsewhere¹³ and will not be discussed further.

Optimizing p38 inhibitors

ATP competitive agents

The p38 inhibitors were initially selected for their anti-inflammatory effect without knowledge of their intracellular target. Once this was identified and structure activity relationships became better understood it was realized the ATP-competitive inhibitors were not selective. Unbiased screens have identified a number of kinases that will bind pyridinyl

imidazole inhibitors similar to SB203580.¹⁴ Furthermore some of these kinases lie within an inflammatory signaling cascade and probably contribute to the biological activity of the small molecule. Furthermore, the IC₅₀s for these “off-target” kinases, such as RIP2,^{14,15} can be below that for p38 α . In such cases these non-selective actions may be beneficial. Given the necessary conservation of key aspects of the ATP-binding pocket amongst the kinome it is inevitable that 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 targets.¹⁶ Ensuring that a particular biological property of a small molecule inhibitor is truly the result of on-target effects can be achieved using a chemical genetic approach. Following the crystallization of p38 α in complex with a pyridinylimidazole or with ATP, it became apparent that the gatekeeper region made intimate contact with the inhibitor but not ATP.⁹ This differential dependence on the gatekeeper and its surrounding hydrophobic pocket meant that it was possible to substitute key residues without altering the kinetics of the kinase.¹⁷ However, such substitutions increased the IC₅₀ of pyridinylimidazole inhibitors by 4 orders of magnitude. This observation presaged one of the earliest examples of the use of chemical genetics to identify the molecular target of small molecule kinase inhibitors.¹⁷ More recently a substitution of Thr106 for Met has been “knocked-in” to the murine p38 α or p38 β alleles.¹⁸ This allows an in-vivo assessment of the target of ATP-competitive antagonists since if the biological action is lost in the knock-in mouse it is the direct result of inhibition of the targeted p38 isoform; whilst if the action is not lost it is likely to be due to the inhibition of another kinase. These mice have been used to show that the ability of SB203580 to reduce myocardial infarction size or inflammatory arthritis is the result of the inhibition of p38 α .^{4,18}

Targeting other regions of the kinase

Given that the constraints of ATP binding and hydrolysis limit diversity within the ATP binding pocket of competent kinases, greater selectivity is often sought by targeting additional features at the edge of this pocket. One such feature is the Asp-Phe-Gly (DFG) motif where the side chain of the Phe169 residue is usually deeply buried in a groove formed between the two lobes of the kinase. One face of the Phe side chain binds to BIRB796, the first in a class of allosteric inhibitors that stabilizes a “DFG out” conformation that is catalytically inactive.¹⁰ In kinase screens BIRB796 is more selective than classic ATP-competitive inhibitors and has a different repertoire of off-target kinases.¹⁶ Consequently, it has been suggested the biological actions of BIRB796 and SB203580 can be compared to confirm p38 as the relevant target.¹⁶ The design of p38 inhibitors continues to evolve in search of greater potency and selectivity. The key features utilized in design are DFG binding, largely to increase potency since this motif is highly conserved among kinases; the small Thr106 gatekeeper, which also confers selectivity since it is found in less than 20% of kinases and Gly110 adjacent to the linker Met109, which also confers selectivity occurring in less than 10% of kinases and can alter its orientation (see 5 for a review).

Other strategies to inhibit p38

As outlined below the ATP competitive antagonists and the allosteric inhibitors have been examined in clinical trials but as yet none has progressed beyond phase 2.¹⁹ Although only a portion of the data is in the public domain most programs seem to have been terminated by an adverse side-effect profile. It is possible that these effects are the result of inhibition of p38 catalytic activity and that optimization requires knowledge of the diverse mechanisms of p38 activation under varied circumstances. Such knowledge may then enable circumstance specific inhibition. The archetypal mode of p38 activation is through a canonical cascade of kinases that terminate in the dual specificity kinases capable of phosphorylating both Thr180 and Tyr182 within the “TGY” activation motif. Whilst these

upstream kinases could be targeted, perhaps of greater interest are the varied substrates downstream of p38 and more recherché mechanisms of activation.

Interestingly there is now widespread evidence that p38 can autophosphorylate the TGY motif under defined circumstances.²⁰ During myocardial ischemia and some other stresses this is the result of its binding a scaffold protein known as TAB1.^{21–24} One strategy that would result in greater selectivity than blanket inhibition of p38 kinase activity would be to disrupt the recognition between p38 and TAB1. This could be achieved by mapping the key features responsible for the interaction or by understanding the natural mechanisms responsible for its regulation.²⁵ Another mechanism that leads to autoactivation is by transphosphorylation of Tyr 323.²⁶ At present it is unclear whether this mechanism, initially identified in lymphocytes, is relevant to the heart.

Adverse events with p38 inhibition

The difficulty in making a complete assessment of the toxicology of p38 inhibitors is the lack of information in the public domain. Nonetheless this topic has been reviewed extensively.^{6–8} Despite diverse structural backbones and at least 2 different mechanisms of inhibition, skin, liver and neurological side effects have curtailed the progression of a number of programs.^{6–8} It is not clear if these effects are the result of off- or on- target actions. However, there seems little doubt that p38 function is of importance to normal cellular homeostasis since it is ubiquitously expressed, highly evolutionary conserved and ablation of the α isoform alone results in early embryonic lethality. Furthermore, circumstances undoubtedly exist where p38 activation benefits the heart.²⁷

Early phase clinical trials with p38 inhibitors

Interrogation of ClinicalTrials.gov with “p38” as the keyword reveals approximately 30 trials that are ongoing or recently completed. The majority of these are for rheumatoid arthritis, neuropathic pain or multiple myeloma. The inhibitors have been most extensively investigated in rheumatoid arthritis where they have so far demonstrated inferior efficacy, and perhaps poorer tolerability, than established therapies.²⁸ In terms of trials of p38 inhibition in the cardiovascular arena these have been limited to small detailed pathophysiological studies thus far 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).²⁹ The salient finding was a suppression of the elevation in CRP and other inflammatory markers (monocytes, neutrophils and total white blood cell count) for 3–4 days post procedure. Oral dosing with the inhibitor (5–40mg, once per day) was for 5 days starting the day before the procedure and was well tolerated.²⁹ In a study of a similar design SB681323 at a dose of 7.5 mg twice per day was compared to placebo in 74 patients with 1:1 randomization (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.³⁰ There were also reductions in other inflammatory markers such as myeloperoxidase, IL6 and IL8. The study was not powered to look at clinical endpoints but there was a significant reduction in episodes of angina albeit on a post-hoc analysis.³⁰

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 not reported and examined 2 dosing regimens with Losmapimod at 7.5mg daily or 7.5mg twice daily compared with placebo. The BMS study is ongoing with a target of 70 patients dosed

with BMS582949 at 100mg daily 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.

Perhaps the most interesting and ambitious study is in the setting of non-ST Elevation Myocardial Infarction (NSTEMI) by GlaxoSmithKline (with Losmapimod at 7.5mg twice daily with or without a 15mg loading dose for 12 weeks compared to placebo, 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 measures of efficacy such as alterations in CRP and measures of infarct size based on biomarkers. In addition there is a sub-study in which changes in infarction and cardiac function will be analyzed by MRI. The study is due to complete in early 2011.

Conclusions

The activity of a large number of kinases adversely affects the injured heart (see table 1). In many cases bioavailable molecules with favorable pharmacodynamic/kinetic properties that selectively inhibit these kinases are not available. However such molecules targeting p38 α and p38 β have been widely available for the last 10 years and are beginning to be examined in patients with ischemic heart disease. As the small molecules to inhibit these additional candidate kinases develop, we look forward to them following the p38 inhibitors as first-in-class.

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Table 1

Additional protein kinases (non-receptor) implicated in heart disease.

#	Abbreviation	Long name or secondary name
1	Akt1/2/3	A.K.A. Protein kinase B (PKB)
2	ASK1	Apoptosis signal-regulating kinase
3	AMPK	AMP activated protein kinase
4	Bmx	A.K.A. Etk (Tec family member)
5	CaMKII	Camodulin-dependent kinase II
6	Cdk9	Cyclin-dependent kinase 9
7	DYRK1A	DYRK1A
8	ERK1/2	Extracellular signal-regulated kinase genes 1 and 2
9	ERK5	Extracellular signal-regulated kinase 5 (A.K.A. BMK1)
10	FAK	Focal adhesion kinase
11	GRK2/5	G-protein-coupled receptor kinase
12	GSK-3 α/β	Glycogen synthase kinase-3 α and β genes
13	JNK1/2	c-jun N-terminal kinase 1 and 2 genes
14	Jak1/2	Janus kinase 1 and 2 genes
15	ILK-1	Integrin-linked kinase 1
16	Lats2	Lats2
17	LKB1	LKB1
18	MEK1/2	Mitogen-activated protein kinase kinase 1 and 2 genes
19	MEK4	Mitogen-activated protein kinase kinase 4
20	MEK5	Mitogen-activated protein kinase kinase 5
21	MEK3/6	Mitogen-activated protein kinase kinase 3 and 6 genes
22	MEK7	Mitogen-activated protein kinase kinase 7
23	MK2	MAPK-activated protein kinase 2
24	mTOR	mTOR
25	Mst1	Mammalian sterile 20-like kinase
26	p70S6K	70 kDa ribosomal protein S6 kinase
27	p90RSK	90-kDa ribosomal S6 kinase
28	Pak1	p21 activated kinase
29	PDK1	phosphoinositide-dependent protein kinase-1
30	PKG1	cGMP-dependent protein kinase type I
31	PKA	Protein kinase A
32	PKC $\alpha/\beta/\gamma$	Protein kinase C (conventional isoforms $\alpha/\beta/\gamma$ genes)
33	PKC δ/ϵ	Protein kinase C (novel isoforms δ/ϵ genes)
34	PKD	Protein kinase D
35	Pyk2	Pyk2
36	Raf-1	Raf-1
37	ROCK1/2	Rho-kinase 1 and 2 genes

#	Abbreviation	Long name or secondary name
38	SGK1	Serum and glucocorticoid-inducible kinase 1
39	Src	Src
40	TAK1	TGF β -activated protein kinase