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G protein-coupled Receptor Kinase 2 as a Therapeutic Target for Heart Failure

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Abstract

An ever-increasing number of people world-wide are developing and suffering from heart failure, and existing therapies, although improved are not ideal. Therefore, innovative treatment strategies are urgently needed. As our understanding of the underlying dysfunction of the myocardium increases, so does our ability to target the mechanisms responsible for heart failure progression. In this review we discuss novel molecular therapies and approaches for the treatment of heart failure. We will focus on the G protein-coupled receptor kinase GRK2, an increasing target for heart failure therapy, based on its important role in disease progression and the therapeutic potential of GRK2 inhibitors.

Introduction

Heart failure (HF) is a leading cause of mortality and morbidity, affecting more than 5 million Americans and representing a growing burden on public health with untold healthcare costs. HF is the common endpoint for several co-morbid conditions, including chronic hypertension or ischemic and valvular heart disease and, most notably, myocardial infarction (MI). Following cardiovascular stress/injury there is an initial compensatory increase in catecholamine production by the sympathetic nervous system (SNS) to enhance inotropy and chronotropy in compromised myocardium; however, it is well recognized that over time, this constant stimulation of the heart can lead to the progressive structural remodeling and myocyte dysfunction characteristic of HF.[1,2] While significant advances have been made in the pharmacological and clinical treatment of HF, these advances offer superficial benefits of symptom management and do not target the causes of cardiac deterioration. In fact, most therapies are aimed at blocking the noxious effects of the hyperactivity of the SNS and other neuro-hormonal imbalances. Pharmacotherapy with β -

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blockers, angiotensin-converting enzyme (ACE) inhibitors, and diuretics aid in the maintenance of blood pressure and systemic circulation, while device therapies such as cardiac resynchronization and left ventricular assist devices (LVADs) promote electrophysiological stability and proper conduction in the myocardium. Despite these advances, the 5 year survival rate for HF patients has remained at 50%. [3] Thus, new therapeutic strategies are urgently needed and targeting the molecular mechanisms involved in cardiac decompensation during HF represents a novel means to inhibit progressive cardiac deterioration and prevent the development of HF.

The complex pathology of HF causes a variety of key signaling pathways, notably G protein coupled receptor (GPCR) signaling, to become dysregulated. [4–6] A crucial mechanism that regulates GPCR signaling is phosphorylation of ligand-bound receptors via GPCR kinases (GRKs). GRKs initiate a process, termed receptor desensitization, via phosphorylation of target GPCRs leading to β -arrestin recruitment, and ultimately receptor internalization and down-regulation. [7–10] In failing human hearts, GRK expression and activity are elevated. In particular, the most abundant GRK in the heart, GRK2, is upregulated, leading to increased phosphorylation, desensitization and down-regulation of key GPCRs, including β adrenergic receptors (β ARs). Genetic manipulation of GRK2 has demonstrated the profound effects of this GRK on cardiovascular function. [11–22] Cardiac overexpression of GRK2 leads to a loss of inotropic reserve and a propensity for HF, especially after ischemic injury. [11,18,23,24] Conversely, inhibition of GRK2 or loss of its expression enhances cardiac function and prevents adverse remodeling after injury. [13–17,19–21] Therefore GRK2 is critical for normal and pathophysiological myocardial signaling and function, and represents a significant target for HF therapy. [8,12,22,23,25–31] There are no current therapies that favorably affect long-term outcomes in end-stage HF, highlighting the need for novel treatment modalities. The purpose of the present review is to reflect on the success and limitations of current preclinical GRK2-targeted therapeutic strategies, as well as discuss new translational avenues that are being pursued.

GRK2 Alterations in the Failing Heart

One mechanism by which the initial compensatory increase in SNS activity leads to cardiac dysfunction is via a reduction in β AR density in the sarcolemmal membrane. As introduced above, in normal myocardium epinephrine or norepinephrine binding to β ARs leads to activation of heterotrimeric G proteins, resulting in dissociation of $G\alpha$ and $G\beta\gamma$ for interaction with and stimulation of downstream signaling cascades. [4,7,26,27,32] The association of GRK2 with dissociated $G\beta\gamma$ results in translocation of GRK2 to the membrane where it phosphorylates ligand-bound GPCRs. [8,9] Phosphorylation of β ARs leads to receptor desensitization and internalization through β -arrestin recruitment and clathrin-mediated endocytosis, followed by degradation or recycling. [7,8] In failing myocardium, the increase in circulating catecholamines and stimulation of β ARs triggers increased expression and activity of GRK2, leading to enhanced phosphorylation, desensitization, and downregulation of β ARs resulting in uncoupled signaling and impaired inotropic response. [1,2] This vicious cycle in HF of increased catecholamine release and decreased β AR density, along with increased GRK2 levels and activity, results in the continual deterioration in myocyte contractile force (Figure 1).

An elevation in cardiovascular levels of GRK2 has been demonstrated in several models of cardiovascular disease, including hypertension,[33] myocardial ischemia,[34] and ventricular hypertrophy,[10] in addition to chronic HF. Evidence for the role of GRK2 in the deterioration of cardiac function during HF was first demonstrated by the generation of transgenic mice with cardiac-specific overexpression of GRK2. In these mice GRK2 expression was elevated to the same degree observed in rodent models of HF as well as human HF patients (3–4 fold).[11,12,23,29,35–37] This increase in GRK2 expression and activity resulted in suppression of catecholamine-stimulated left ventricular (LV) contractility and function.[11] Reciprocally, transgenic mice expressing an inhibitor of GRK2 (β ARKct – see below) resulted in animals with enhanced cardiac contractility and a super-sensitivity to catecholamine stimulation.[11] In addition, although global GRK2 knockout (KO) mice were not born,[30] heterozygous GRK2 KO mice with 50% less GRK2 expressed in myocytes (and all cells) resulted in enhanced cardiac function and increased inotropic reserve *in vivo*. [14] Together, these studies demonstrate the importance of GRK2 in normal and pathophysiological cardiac function, and highlight the potential for GRK2 inhibition in cardiovascular diseases such as HF.

GRK2 Targeting and Inhibition in the Failing Heart

To initially determine the therapeutic potential of GRK2 inhibition in the treatment of HF, mice with cardiac-specific expression of a carboxyl-terminal peptide of GRK2, β ARKct, were generated.[11] This C-terminal peptide portion of the pleckstrin homology domain of GRK2 is critical for association with $G\beta\gamma$ and translocation to the sarcolemmal membrane to enact GPCR phosphorylation. β ARKct expression has been shown to sequester $G\beta\gamma$, inhibiting GRK2-mediated phosphorylation and desensitization of receptors and thereby promoting β AR and cardiac GPCR function in the myocardium (Figure 2).[11,14,38] As introduced above, β ARKct transgenic mice demonstrated enhanced basal and agonist-stimulated LV function *in vivo*, which was virtually identical to heterozygous GRK2 KO mice.[11,14] Interestingly, a cross between the cardiac GRK2 overexpressing mice and the β ARKct line demonstrated the ability of this peptide to overcome the detrimental effects of GRK2 overexpression, enhancing β AR coupling and contractility.[13] Importantly, these studies demonstrated the ability of β ARKct to inhibit GRK2 function *in vivo*, even when there are elevated levels demonstrated in HF. Moreover, loss of GRK2 activity towards GPCRs in the heart improves cardiac function.

Further proof of the therapeutic potential of GRK2 inhibition and β ARKct, in particular, came from experimental crosses of β ARKct transgenic mice with various mouse models of HF. The first was a model of murine dilated cardiomyopathy through knockout of the muscle LIM protein (MLP^{-/-}). The LV enlargement and deterioration of function demonstrated in the MLP^{-/-} animals was completely abolished in MLP^{-/-}/ β ARKct mice. [14] β ARKct also improved contractility and restored β AR sensitivity, demonstrating the ability of this peptide inhibitor to prevent cardiomyopathy *in vivo*. Transgenic mice overexpressing the sarcoplasmic reticulum Ca²⁺-binding protein calsequestrin (CSQ) were studied as a second model. CSQ mice represented a severe model of dilated cardiomyopathy with a significantly shortened survival. A cross between this line and the β ARKct transgenic line demonstrated prolonged life, with significantly reduced cardiac dilation accompanied by

enhanced LV function.[15] Importantly, treatment of CSQ/ β ARKct mice with the β AR blocker metoprolol further potentiated survival rate, demonstrating synergy in these two treatment modalities. While β ARKct inhibits β AR downregulation by decreasing GRK2 translocation to the receptor, the addition of a β AR blocker may further preserve β AR density by decreasing agonist-mediated receptor activation and may contribute to an inhibition of detrimental signaling downstream of β AR activation. Thus, receptor density is maintained at normal levels so when catecholamines do increase enough in the myocardium for needed inotropic reserve there is efficient signaling. The fact that there was this additional effect with the two treatment modalities could also mean that the β ARKct has non- β AR effects, which has been found since this study (see this review).

In a third model demonstrating a cross between a mouse model of hypertrophic cardiomyopathy (HCM) and β ARKct, β ARKct expression decreased both cardiac remodeling and hypertrophic gene expression in addition to improving cardiac function and exercise tolerance.[6] These data demonstrate a role for GRK2 inhibition in the therapeutic treatment of both hypertrophic and dilated cardiomyopathy for the prevention of HF.

The above studies provide strong evidence for a therapeutic role of the β ARKct peptide in the prevention of HF despite the etiology; however, whether this is primarily due to inhibition of GRK2 translocation or potentially other signaling mechanisms was unclear. To better elucidate the role of GRK2 in the development of cardiac dysfunction and HF required the use of GRK2 KO animals. As mentioned above, conventional GRK2 KO proved embryonic lethal;[30] therefore, two conditional knockout lines were developed, α MHC-Cre/GRK2^{flox/flox} and MerCreMer/GRK2^{flox/flox}, allowing for KO of GRK2 developmentally after birth or upon tamoxifen treatment, respectively.[16] Using a mouse model of coronary artery ligation to induce MI, Raake *et al* demonstrated that GRK2 ablation prior to injury prevented maladaptive cardiac remodeling and preserved β AR function. Furthermore, GRK2 ablation 10 days post injury terminated LV remodeling and was able to preserve cardiac contractility and improve survival.[16] It should be noted that use of an early pan-Cre in GRK2 floxed mice recapitulated embryonic lethality and also use of Nkx2.5-Cre, deleting GRK2 in myocytes before birth, resulted in mice that had some adverse supersensitivity to chronic isoproterenol treatment.[39] However, the data in induced GRK2 KO after HF was established post-MI [16] is the most compelling evidence of a causal role for GRK2 in the maladaptive progression of cardiac remodeling and dysfunction leading to HF. Importantly, in this study there was also an arm of β AR blockade with and without GRK2 deletion in myocytes where the loss of GRK2 proved to be more effective at improving post-MI cardiac function and reversing remodeling and the two together gave the same positive effect of GRK2 KO alone.[16] Thus, ample data exists, giving further credence to targeting GRK2 inhibition for the treatment of HF and also, importantly, supporting the idea that β ARKct prevents failure primarily through its role in GRK2 inhibition.

GRK2 Targeting in Acute Cardiac Injury

Acute ischemic events are known precursors of MI, and are a major cause of mortality in HF patients.[40] During an ischemic event, insufficient blood flow, and thus oxygen supply,

leads to myocyte cell death that expands with prolonged ischemia.[41] Reperfusion of ischemic regions increases reactive oxygen species (ROS) production and Ca^{2+} levels resulting in further cell death, myocardial stunning, increased risk of cardiac arrhythmia, micro-vascular obstruction and fibrosis.[6,17,19,42,43] Increased GRK2 expression and activity is known to play a role in HF progression; therefore, studies were performed to determine if GRK2 is a regulator of acute ischemic injury. In a model of ischemia/reperfusion (I/R), mice overexpressing GRK2 exhibited increased infarct size and apoptosis post-injury due to reduced induction of vital cell survival pathways (i.e. Akt, NO generation).[17] Further, a recent study from our lab has determined that GRK2 exerts these detrimental effects via translocation to mitochondria, an event dependent on both ERK-mediated phosphorylation and heat shock protein chaperone function.[18] Taken together, these studies suggest that increased GRK2 expression and activity are detrimental in both acute and prolonged cardiac injury to the myocardium. To determine whether GRK2 represents a viable therapeutic target in I/R, the extent of injury was determined in two lines of GRK2 KO mice (global and inducible GRK2 KO, with induction prior to injury). Both GRK2 KO lines exhibited improved cardiac function and activation of pro-survival pathways (Akt, Bcl-2, Bcl-xl) post-injury, which led to smaller infarct size and decreased apoptosis and caspase 3,9 activity.[19] In addition, GRK2 KO markedly decreased cytochrome C release, a key measure of mitochondrial-dependent apoptosis in myocytes. [19]

To further investigate the therapeutic potential of GRK2 inhibition during ischemic injury, the cardiac-specific β ARKct line was exposed to sham or MI surgery. Similar to GRK2 KO, β ARKct mice exhibited improved survival and cardiac performance after MI.[17] In addition, β ARKct significantly decreased infarct size and apoptosis relative to control or GRK2 overexpressing mice.[17] Thus, GRK2 inhibition appears to be a powerful cardioprotective strategy and more importantly, shows that the increased GRK2 expression early after myocardial injury is maladaptive from the “get go” and detrimental to cardiac cell survival and function.

Targeting GRK2 in HF via Gene Therapy Using β ARKct

Although β ARKct yielded promise in treating various cardiac insults using transgenic mice, it was necessary to develop a means to achieve effective delivery of β ARKct to its desired site of action, the heart, in animals and eventually in humans. Gene therapy modalities were undertaken and original studies were done using adenoviral vectors carrying the β ARKct. For example, in a rabbit model of HF, adenovirus-mediated delivery of β ARKct led to marked reversal of ventricular dysfunction and improvement of cardiac function post-injury. [20] Since adenoviral vectors cannot support chronic transgene expression needed for HF therapy, β ARKct was recently cloned into adeno-associated viral vectors (AAV) and studies with AAV6 were started since this serotype has shown some muscle-tropism. Importantly, direct myocardial injection into rats 12 weeks after sham or MI surgery resulted in long-term expression of β ARKct and cardio-protection, including a reversal of LV remodeling, increased contractile performance, and normalized β AR signaling.[21] Of note, co-administration of metoprolol offered no therapeutic benefit beyond AAV6- β ARKct expression.[21] This is in contrast to our previous data demonstrating a synergistic effect

upon administration of metoprolol in the CSQ/ β ARKct mice. However, in the CSQ model this was only on survival of this severe HF phenotype. This discrepancy may be due to a difference in the severity of the disease model or the degree of inhibition achieved by AAV6- β ARKct delivery to the failing rat heart. Importantly, like the GRK2 KO mice described above, limiting GRK2 activity in the failing heart alone produces a greater benefit than β AR blockade and at least all studies using both modalities show they are compatible, which increases potential future uses of GRK2 inhibitors. Mechanistically, the use of each modality and their use together is not completely understood; however, our most recent studies would argue for GRK2 inhibition having broader effects than only on β ARs.

The development of AAV6- β ARKct provided the means for cardiac-specific delivery and prolonged inhibition of GRK2 *in vivo*, allowing for administration of this therapeutic in large animal models of disease and the progression of preclinical trials. This is both relevant and necessary since larger mammals (i.e. dogs, pigs) possess cardiovascular systems that more closely resemble that of humans and have been used to successfully study gene therapy targets.[44,45] Therefore, a porcine model of ischemic cardiomyopathy-induced HF was utilized to observe the effect of administration of either AAV6- β ARKct or control AAV6-luciferase two weeks post injury. The vectors were safely and efficiently delivered via retrograde injection into the anterior interventricular vein, thereby targeting the myocardium with no expression observed in non-targeted tissues.[46] In addition, AAV administration did not alter survival compared to sham controls. In agreement with prior findings, β ARKct improved cardiac function and preserved β AR responsiveness, observed via echocardiography and hemodynamics 6 weeks post MI.[46] Furthermore, β ARKct reversed LV remodeling, decreasing LV mass, collagen deposition, and fetal gene expression compared to control animals. This study demonstrated for the first time that β ARKct administration in a large animal model of HF was cardioprotective, supporting the notion of β ARKct as a clinical therapeutic. While AAV6- β ARKct administration was able to significantly enhance cardiovascular outcomes post-MI, this therapy did not restore cardiac function to baseline, suggesting that β ARKct may need to be used in combination with other treatments to yield complete recovery. In addition, the terminal endpoint in this study was 6 weeks post MI; therefore, future studies will need to address the extent of β ARKct-mediated cardioprotection over prolonged periods of time.

Adrenal GRK2 Inhibition as a Sympatholytic in HF

In the body, catecholamine production via the SNS and secretion occurs at the level of the sympathetic synapse as well as in the adrenal medulla. We have found that GRK2 also plays a role at the level of the SNS in HF, as like in failing myocardium, GRK2 is up-regulated in the adrenal gland of HF animals.[47] The target for this increased GRK2 activity are α_2 -ARs, which plays a role in catecholamine secretion as it is the sole inhibitory presynaptic autoreceptor, thereby inhibiting catecholamine release.[48] Accordingly, the α_2 -ARs, like β ARs in failing myocardium, are uncoupled and down-regulated in the adrenal gland of HF animals via GRK2.[47] Inhibition of adrenal GRK2 through expression of β ARKct was found to restore α_2 -AR signaling and lower catecholamines in HF animals and this alone was able to improve the function of the failing heart.[47] These findings were also confirmed in SNS-targeted GRK2 KO mice where loss of GRK2 in cells that synthesize

epinephrine resulted in lower circulating catecholamines after cardiac injury and this sympatholytic effect lead to improved heart function and preserved cardiac dimensions post-MI.[49] These data suggest that GRK2 inhibition in the adrenal gland may be a therapeutic strategy to combat the detrimental effects of HF acting as a novel sympatholytic to decrease catecholamine levels. Therefore, inhibiting GRK2 in more than cardiac myocytes may be beneficial in HF and this appears to support the use of a more global GRK2 inhibitory strategy, one that could be attained with small molecule pharmacological inhibitors, which as discussed below are on the horizon.

Small Molecule Inhibition of GRK2

Increasing interest is being placed in the development of small molecule inhibitors of GRK2 for the treatment of cardiac dysfunction and HF. The Blaxall laboratory recently described the novel small molecule G $\beta\gamma$ inhibitors M119 and gallein, which demonstrated GRK2 inhibition *in vitro* and *in vivo* via binding the protein interaction domain of G $\beta\gamma$ thereby decreasing G $\beta\gamma$ -GRK2 interactions and GRK2 membrane translocation.[50] Further, these compounds exhibit a similar prevention of HF development to that seen with cardiac β ARKct expression in the CSQ murine HF model.[50] With increasing attention being paid to the repurposing of drugs, recent work from the Tesmer laboratory provides an additional example of how this approach may lead to beneficial new therapeutics for HF, specifically regarding GRK2 inhibition. In this study, a selective serotonin reuptake inhibitor (SSRI) paroxetine was shown to inhibit GRK2 with selectivity over other GRK subfamilies.[51] In isolated adult mouse ventricular myocytes, paroxetine pretreatment significantly potentiated the isoproterenol-mediated increase in contractility without altering baseline function. More importantly, using hemodynamic measurements in wild-type mice, paroxetine pretreatment demonstrated a small increase in baseline contractility and a significant increase in isoproterenol-mediated inotropy *in vivo*. [51] Unlike paroxetine, the SSRI fluoxetine did not demonstrate GRK2 inhibition during *in vitro* kinase assays and exhibited no effect on myocyte contractility *in vitro* or *in vivo*. [51] Therefore, it will be interesting to see if paroxetine may have HF modulatory properties in animal models or whether any GRK2 inhibitory activity is seen in human HF patients taking this drug for depression (Figure 3).

It is evident that the use of a pharmacologic inhibitor of GRK2 may prove more reasonable in translation in HF over β ARKct-mediated gene therapy. However, cardiac-specific GRK2 inhibition still may prove to be the most efficacious for HF. Importantly, both avenues are currently being actively explored with a β ARKct trial being planned in HF and small molecule GRK2 inhibitors being sought. A small molecule would also have the advantage of inhibiting adrenal GRK2 to lower catecholamine secretion, which could significantly aid in HF improvement. With the emerging exciting data with paroxetine, structure-based drug design using paroxetine as the model may also generate new compounds with increased selectivity and potency for GRK2 inhibition over SSRI activity and provide lead compounds to be tested. Furthermore, it is important to consider that any small molecule inhibitor of GRK2 developed as a human HF therapy would have to demonstrate a benefit over the current standard of care, namely β AR-blocker treatment. The multi-faceted functions of GRK2 and implications for cell function and disease have been reviewed in detail.[22,52,53] In addition to the inhibition of adrenal GRK2 that would lower catecholamine secretion and

thereby potentially enhance the observed efficacy of beta-blocker therapy, the ubiquitous expression of GRK2 and its numerous roles throughout the body highlight the potential benefit of this treatment modality. GRK2 has been demonstrated to play a central role in immune cell activation and migration[54] and increasing evidence suggests a critical role for GRK2 in cell death[55], such that global GRK2 inhibition may decrease myocardial remodeling in addition to restoring β AR sensitivity. To this end, our lab is actively investigating the effects of GRK2 inhibition on cardiomyocyte survival and myocardial remodeling during ischemic injury.

Conclusions

The increasing prevalence of HF, with mounting healthcare costs, underlines the importance of determining alternative approaches and novel treatments. In this review, we discussed the mounting evidence in support of inhibition of GRK2 as a therapeutic target in HF. In developing treatments to combat the deleterious effects of GRK2 up-regulation and activity during HF, specificity will be a key point of discussion. GRK2 is ubiquitously expressed and is highly expressed in non-cardiac tissues (i.e. brain, spleen, leukocytes), with known key regulatory functions such as in immune cell migration.[22,31] This is of particular interest in the development of small molecular inhibitors of GRK2, where off-target effects may be a concern. Despite promising results utilizing both AAV- β ARKct and small molecule administration, several questions remain regarding the therapeutic potential of these interventions. Further large animal and preclinical trials will need to determine the therapeutic window for clinical treatment in all potential target conditions, including acute ischemic events, MI, hypertrophy, and HF. The studies reviewed here also suggest that further analysis of the role of GRK2 in different etiologies of cardiac dysfunction may shed light on additional targets or methods of intervention. This research may pave the way for novel GRK2-based approaches for the prevention of cardiovascular remodeling and HF.

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Figure 1. β AR signaling in normal myocardium and during heart failure

In normal myocardium (left), norepinephrine or epinephrine (NE, Epi) binding to β_1 ARs leads to activation and dissociation of the heterotrimeric G protein complex. $G_{\alpha s}$ then directly stimulates adenylyl cyclase (AC) at the membrane leading to cAMP production and PKA activation for downstream signaling. Concurrently, GRK2 associates with dissociated $G\beta\gamma$ subunits at the membrane for translocation and subsequent phosphorylation of the agonist-bound receptor. Phosphorylation by GRK2 leads to the recruitment of β -arrestins, resulting in receptor desensitization and subsequent internalization. In the HF condition (or after a cardiac insult), GRK2 expression and activity are elevated leading to increased phosphorylation of agonist bound GPCRs and enhanced β -arrestin recruitment, resulting in receptor down-regulation through enhanced internalization and this contributes to the lack of inotropic reserve seen in failing myocardium and also to other untoward effects of elevated GRK2.

Figure 2. β ARKct-mediated inhibition of sympathetic nervous system over-activity during heart failure

During HF, increased circulating catecholamines bind to β ARs in the myocardium and cooperate with enhanced GRK2 activity to enact significant receptor down-regulation and decreased cardiac function. Cardiac specific expression of β ARKct competes with endogenous GRK2 for association with $G\beta\gamma$, resulting in decreased membrane translocation of GRK2 to the receptor. This results in normalization of β AR signaling in the failing myocytes and through this re-gained responsiveness, SNS activity is lowered and cardiac function can be improved.

Figure 3. Paroxetine-mediated preservation of cardiac function during HF

Inhibition of GRK2 activity via delivery of this small molecular inhibitor (or any future pharmacologic inhibitor) of this enzyme decreases GRK2-mediated GPCR phosphorylation. GRK2 is still translocated to the membrane and activated β AR, however its catalytic activity is inhibited allowing for inhibition of desensitization and restoration of impaired signaling.