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Pharmacological and Activated Fibroblast Targeting of Gβγ-GRK2 After Myocardial Ischemia Attenuates Heart Failure Progression

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ABSTRACT

BACKGROUND Cardiac fibroblasts are a critical cell population responsible for myocardial extracellular matrix homeostasis. Upon injury or pathological stimulation, these cells transform to an activated myofibroblast state and play a fundamental role in myocardial fibrosis and remodeling. Chronic sympathetic overstimulation, a hallmark of heart failure (HF), induces pathological signaling through G protein βγ (Gβγ) subunits and their interaction with G protein–coupled receptor kinase 2 (GRK2).

OBJECTIVES This study investigated the hypothesis that Gβγ-GRK2 inhibition and/or ablation after myocardial injury would attenuate pathological myofibroblast activation and cardiac remodeling.

METHODS The therapeutic potential of small molecule Gβγ-GRK2 inhibition, alone or in combination with activated fibroblast- or myocyte-specific GRK2 ablation—each initiated after myocardial ischemia–reperfusion (I/R) injury—was investigated to evaluate the possible salutary effects on post-I/R fibroblast activation, pathological remodeling, and cardiac dysfunction.

RESULTS Small molecule Gβγ-GRK2 inhibition initiated 1 week post-injury was cardioprotective in the I/R model of chronic HF, including preservation of cardiac contractility and a reduction in cardiac fibrotic remodeling. Systemic small molecule Gβγ-GRK2 inhibition initiated 1 week post-I/R in cardiomyocyte-restricted GRK2 ablated mice (also post-I/R) still demonstrated significant cardioprotection, which suggested a potential protective role beyond the cardiomyocyte. Inducible ablation of GRK2 in activated fibroblasts (i.e., myofibroblasts) post-I/R injury demonstrated significant functional cardioprotection with reduced myofibroblast transformation and fibrosis. Systemic small molecule Gβγ-GRK2 inhibition initiated 1 week post-I/R provided little to no further protection in mice with ablation of GRK2 in activated fibroblasts alone. Finally, Gβγ-GRK2 inhibition significantly attenuated activation characteristics of failing human cardiac fibroblasts isolated from end-stage HF patients.

CONCLUSIONS These findings suggested consideration of a paradigm shift in the understanding of the therapeutic role of Gβγ-GRK2 inhibition in treating HF and the potential therapeutic role for Gβγ-GRK2 inhibition in limiting pathological myofibroblast activation, interstitial fibrosis, and HF progression. (J Am Coll Cardiol 2017;70:958–71)

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Heart failure (HF) is a devastating disease characterized by interstitial fibrosis, chamber remodeling, and reduced ventricular compliance. Cardiovascular disease remains the predominant cause of mortality in the United States, presenting a considerable economic burden, with estimated annual direct and indirect costs totaling approximately $320 billion (1). Regardless of etiology, HF generally involves adverse myocardial remodeling characterized by excessive deposition of extracellular matrix proteins by pathologically activated cardiac fibroblasts; this reduces tissue compliance, promotes arrhythmogenesis, and accelerates HF progression (2). Despite the critical importance of fibrosis in HF, there are essentially no clinical interventions that effectively target the cardiac fibroblast nor its pathological contributions to disease progression.

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The adrenergic system plays a fundamental role in the physiological regulation of the myocardium; however, chronic stimulation can induce cardiac hypertrophy and fibrosis, which are important components of HF pathophysiology (3). Stimulation of the β-adrenergic receptor (β-AR) induces conformational changes in G protein βγ (Gβγ) subunits, ultimately resulting in activation and membrane recruitment of G protein–coupled receptor kinase 2 (GRK2) (4). Expression of GRK2 is known to be elevated in patients with HF (4), and recent studies have suggested that this is associated with β-AR uncoupling and downregulation in fibroblasts, which can promote a pro-fibrotic phenotype (5).

We and others have explored several approaches to specifically target GRK2 and its interaction with Gβγ subunits. These efforts have demonstrated the therapeutic potential of Gβγ-GRK2 inhibitory peptides (6) or compounds (7–9). The beneficial effects of the small molecule gallein, which selectively inhibits the interaction between Gβγ and GRK2 (7,10), were recently demonstrated in several animal models of HF (7,8). Although the Gβγ-GRK2 interface represents an important target of therapeutic interventions for HF, the mechanisms and therapeutic potential of Gβγ-GRK2 inhibition specifically within cardiac fibroblasts and the progression of fibrosis have yet to be elucidated.

In the present study, small molecule inhibition of Gβγ-GRK2 initiated 1 week after myocardial ischemia–reperfusion (I/R) ameliorated the progression of cardiac dysfunction and pathological cardiac remodeling, particularly regarding infarct expansion. Furthermore, inducible ablation of GRK2 in the pathologically activated cardiac fibroblasts 1 week post-I/R was found to be equally cardioprotective, whereas gallein provided significant cardioprotection in animals with post-I/R ablation of GRK2 in cardiomyocytes. This cardioprotection in vivo correlated with a reduction in the activation state of primary mouse and human HF-derived cardiac fibroblasts when treated with gallein. These data support a paradigm shift in proposed mechanisms behind the protective effects of Gβγ-GRK2 inhibition in the treatment of HF.

ABBREVIATIONS AND ACRONYMS

AR = adrenergic receptor

cAMP = cyclic adenosine monophosphate

GRK2 = G protein–coupled receptor kinase 2

I/R = ischemia–reperfusion

LV = left ventricle

MHC = myosin heavy chain

qPCR = quantitative polymerase chain reaction

SMA = smooth muscle actin

TGF = transforming growth factor

METHODS

We recently reported a possible therapeutic role for interdicting pathological Gβγ-GRK2 binding interactions with the small molecule gallein (8). In the present study, gallein was evaluated for its therapeutic efficacy in a more clinically relevant I/R model of HF; mice were subjected to I/R through coronary artery occlusion, followed by 4 weeks of reperfusion. Gallein administration was initiated 1 week post-I/R at 2.5 mg/kg/day and titrated to a maximum dose of 10 mg/kg/day over 3 weeks, followed by assessment of cardiac function by echocardiography and histological analysis of fibrotic remodeling 4 weeks after injury. To biochemically assess injury severity, transcript expression of fibrotic and HF markers was assessed by quantitative polymerase chain reaction (qPCR).

Conditional cardiomyocyte-targeted GRK2 knockout mice were achieved by crossing $GRK2^{fl/fl}$ animals with mice possessing tamoxifen-inducible Cre recombinase under the control of the endogenous promotor for α-myosin heavy chain ($\alpha\text{-}MHC^{MCM}$) (11). $GRK2^{fl/fl}$ animals were also crossed with mice that expressed inducible Cre recombinase under the control of the endogenous promoter for periostin ($Postn^{MCM}$) (12). Tamoxifen administration via the chow was initiated after surgery and continued for 2 weeks to achieve inducible GRK2 ablation in a cell-specific manner.

Detailed materials and methods are included in the Online Appendix.

RESULTS

Optimum dosing and administration, along with initial therapeutic efficacy of gallein, were first evaluated and established in wild-type C57Bl/6 mice subjected to I/R injury. Gallein treatment conferred substantial protection against myocardial dysfunction and dilation (Online Figures 1A to 1C). Furthermore, examination of collagen deposition by Masson's trichrome