Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 45, No. 11, 2005
ISSN 0735-1097/05/$30.00
doi:10.1016/j.jacc.2005.02.062

Targeted Inhibition of β-Adrenergic Receptor Kinase-1–Associated Phosphoinositide-3 Kinase Activity Preserves β-Adrenergic Receptor Signaling and Prolongs Survival in Heart Failure Induced by Calsequestrin Overexpression

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OBJECTIVES Desensitization and down-regulation of β-adrenergic receptors (βARs) are prominent features of heart failure largely mediated by increased levels of βAR kinase-1 (βARK1).

BACKGROUND β-adrenergic receptor kinase 1 interacts with phosphoinositide-3 kinase (PI3K), and upon agonist stimulation, the βARK1/PI3K complex is recruited to agonist-stimulated βARs. Here we tested the hypothesis that in vivo selective inhibition of βARK1-associated PI3K activity would preserve βAR signaling and, therefore, improve cardiac function and survival in experimental heart failure.

METHODS We used a murine model of heart failure induced by calsequestrin (CSQ) cardiac-specific overexpression; CSQ mice were crossed with mice overexpressing in the heart a catalytically inactive PI3Kγ ($PI3K\gamma_{inact}$) to competitively displace endogenous PI3K from βARK1.

RESULTS Catalytically inactive PI3KγPI3K overexpression in CSQ mice inhibited βARK1-associated PI3K activity, normalized βAR levels, and preserved βAR responsiveness to isoproterenol (ISO). Restoration of βAR signaling via $PI3K\gamma_{inact}$ overexpression resulted in marked improvement of cardiac function and a significant prolongation of survival. Importantly, the effects of $PI3K\gamma_{inact}$ overexpression were restricted to βAR signaling, because cellular PI3K signaling was unaltered, as shown by the similar activation of multiple downstream signaling pathways in both CSQ and CSQ/$PI3K\gamma_{inact}$ mice.

CONCLUSIONS These data in the CSQ model of cardiac dysfunction indicate that membrane-targeted PI3K activity plays a detrimental role in heart failure, and its inhibition represents a novel therapeutic approach to ameliorate cardiac dysfunction and improve survival. (J Am Coll Cardiol 2005;45:1862–70) © 2005 by the American College of Cardiology Foundation

Heart failure is a complex disorder whose prognosis remains poor, despite continuous advances in therapy (1). Clarification of the signaling pathways that promote deterioration of cardiac function is an intense area of research because inhibition of these deleterious signals could serve as a novel therapeutic approach.

Failing human hearts are characterized by extensive abnormalities of the β-adrenergic receptor (βAR) system, including down-regulation and desensitization of βARs resulting in a state of reduced responsiveness to agonist stimulation (2). Interestingly, whether changes in βAR signaling represent an adaptive and protective process, as some postulate (3), or whether βAR dysregulation is actually detrimental (4) is still controversial. Results from our previous studies suggest that chronic βAR dysfunction in the failing heart is maladaptive and contributes to the deterioration in cardiac function (5–7).

Previous studies have shown that agonist-induced βAR dysfunction begins with receptor phosphorylation, followed by rapid uncoupling of the receptor from its cognate G protein (desensitization), and subsequent targeting of the phosphorylated receptor for endocytosis, a process linked to chronic receptor down-regulation (4). Phosphorylation can be mediated by second messenger kinases (for example protein kinase A or protein kinase C), or by a specialized family of G protein-coupled receptor kinases (GRKs) (7,8); GRK2, known commonly as βAR kinase-1 (βARK1), is the most abundant isoform expressed in the heart (9,10). Because βARK1 levels are consistently and markedly elevated in both human (11) and experimental heart failure (5,7,12), up-regulation of βARK1 has been postulated to play an important role in the reduced βAR responsiveness associated with cardiac dysfunction.

Upon agonist stimulation, binding of cytosolic βARK1 to liberated $G_{\beta\gamma}$ subunits facilitates its translocation to the plasma membrane, resulting in the phosphorylation of agonist-occupied receptors (13). We have recently demonstrated that βARK1 forms a cytosolic complex with phosphoinositide-3 kinase (PI3K) (14). Upon agonist stimulation, the βARK1/PI3K complex is recruited to activated βARs (15), wherein generation of D-3 phosphoinositides by PI3K is required for receptor endocytosis (14). In vivo, inhibition of receptor-localized PI3K activity prevents βAR abnormalities

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Manuscript received December 13, 2004; revised manuscript received January 28, 2005, accepted February 14, 2005.

Abbreviations and Acronyms

βAR	= β-adrenergic receptor
βARK1	= βAR kinase-1
cAMP	= cyclic adenosine monophosphate
CSQ	= calsequestrin
GSK	= glycogen synthase kinase 3β
ISO	= isoproterenol
MAPKs	= mitogen-activated protein kinases
PKB	= protein kinase B
PI3K	= phosphoinositide-3 kinase
$PI3K\gamma_{inact}$	= catalytically inactive PI3Kγ
WT	= wild type
%FS	= percent fractional shortening

after catecholamine stimulation or pressure overload, resulting in less deterioration in cardiac function (7). However, whether targeted inhibition of PI3K can rescue βAR abnormalities under conditions of chronic heart failure, and ultimately improve cardiac function and survival, is not known. To test this, we used a murine model of heart failure induced by cardiac-specific overexpression of the calcium-binding protein calsequestrin (CSQ) (16). This model recapitulates human heart failure in many aspects, including the morphological progression from cardiac hypertrophy to dilated cardiomyopathy (16), the high rate of premature death (6), and the sensitivity to beta-blocker therapy (6).

In order to inhibit βAR-localized PI3K activity in failing hearts, we bred the CSQ mice with transgenic mice overexpressing a catalytically inactive PI3Kγ ($PI3K\gamma_{inact}$) that competitively displaces the endogenous enzyme from βARK1, therefore preventing its recruitment to agonist-stimulated βARs. We determined the effects of $PI3K\gamma_{inact}$ overexpression on βAR function and PI3K signaling, and monitored the progression of cardiac dysfunction and the rates of survival of single and binary transgenic mice.

METHODS

Experimental animals. Mice overexpressing CSQ or $PI3K\gamma_{inact}$ were engineered as previously described (7,16). Briefly, the alpha-myosin heavy chain promoter was used to drive cardiac-targeted overexpression of canine cardiac CSQ or a PI3Kγ mutant lacking the adenosine triphosphate-binding domain ($PI3K\gamma_{inact}$). To ensure identical genetic backgrounds, both CSQ and $PI3K\gamma_{inact}$ strains were backcrossed >10 generations onto a dilute brown non-agouti genetic background. F1 pups were generated from the crossbreeding of CSQ transgenic mice with $PI3K\gamma_{inact}$ transgenic mice. Wild type (WT), CSQ transgenic, or CSQ/$PI3K\gamma_{inact}$ transgenic littermates of either gender were used for this study and were handled according to the approved protocols and the animal welfare regulations at Duke University Medical Center.

Membrane fractionation, βAR radioligand binding, and adenylyl cyclase activity. Membrane and cytosolic fractions from left ventricles flash-frozen in liquid N_2 were prepared as described previously (8). Because we have previously shown the K_d of ^{125}I-CYP binding to the βARs in membranes prepared from mouse hearts is 30 pmol/l (17), we used a single saturating concentration of ^{125}I-CYP (250 pmol/l) to assess the total βAR density in the mouse cardiac membranes. Receptor density (fmol) was normalized to milligrams of membrane protein. Adenylyl cyclase assays were performed as described previously (8), using 20 μg of the membrane fraction. Generated cyclic adenosine monophosphate (cAMP) was quantified using a liquid scintillation counter (MINAXI-4000, Packard Instrument Co., PerkinElmer, Boston, Massachusetts).

Immunoprecipitation and immunoblotting. Immunoprecipitation and immunoblotting were performed as described previously (7,8). Detection was carried out using ECL (Amersham Biosciences Corp., Piscataway, New Jersey), and bands were quantified using Bio-Rad (Hercules, California) Flouro-S Multimage software densitometry.

PI3K. Phosphoinositide 3-kinase activity assays were carried out by immunoprecipitation of the PI3Kα and γ isoforms from the cytosolic fraction as described previously (15). βARK1-associated PI3K activity was measured after immunoprecipitation of 400 μg of proteins from the membrane fraction with polyclonal antibody directed against βARK1 (Santa Cruz Biotechnology Inc., Santa Cruz, California).

Mitogen-activated protein kinase activity. Mitogen-activated protein kinase activities were assessed from left ventricular cytosolic extracts as the capacity of immunoprecipitated extracellular signal-regulated kinase (ERK)2-p42/ERK1-p44, c-Jun N-terminal kinase (JNK)1, p38, and p38-beta (Santa Cruz) to phosphorylate in vitro substrates (myelin basic protein and glutathione S transferase [GST]-proto-oncogene c-Jun [cJun]) as previously described (18).

RNA isolation and Northern blotting. RNA was isolated from left ventricles using the RNA-Bee-RNA isolation reagent (Tel-Test Temco Inc., Friendswood, Texas) according to the manufacturer's instructions; 10 μg of RNA were size-fractionated by denaturing formaldehyde gel electrophoresis, transferred to nylon membrane by capillary action, cross-linked with ultraviolet light, and hybridized with a mouse β_1AR ^{32}P-labeled cDNA probe. After hybridization, filters were washed under stringent conditions, and transcripts were detected by autoradiography.

Transthoracic echocardiography. Serial echocardiography was performed on conscious mice with an HDI 5000 echocardiograph (Philips, Böblingen, Germany) at 8, 12, and 16 weeks of age, as previously described (19).

Statistical analysis. Data are expressed as mean values ± SEM. Multigroup comparisons were performed using one-way analysis of variance (ANOVA) with Neuman-Keuls correction. Serial echocardiography results were analyzed by repeated-measures ANOVA. Survival was analyzed by Kaplan-Meier analysis. Hazard ratios for the presence of $PI3K\gamma_{inact}$ transgene and gender were derived from the Cox proportional hazard model using SAS software (SAS Insti-