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PRECLINICAL RESEARCH

Protein Kinase C Inhibition With Ruboxistaurin Increases Contractility and Reduces Heart Size in a Swine Model of Heart Failure With Reduced Ejection Fraction

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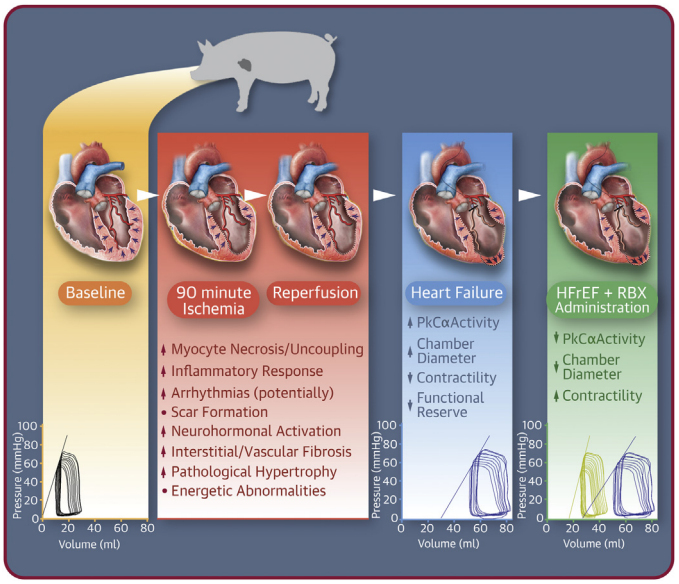
VISUAL ABSTRACT



Sharp, T.E. et al. J Am Coll Cardiol Basic Trans Science. 2017;2(6):669-83.

HIGHLIGHTS

- **Acute protein kinase A α (PKCα) inhibition with ruboxistaurin reduces heart size post-myocardial infarction.**
- **Acute PKCα inhibition with ruboxistaurin increases contractility post-myocardial infarction.**
- **PKCα phosphorylation at Thr638 is positively correlated to increases in left ventricular volumes and reduced ejection fraction, indicative of disease progression.**

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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ABBREVIATIONS AND ACRONYMS

ADHF = acute decompensated heart failure

DIG = digitalis

DOB = dobutamine

ECG = electrocardiogram

EDPVR = end-diastolic pressure-volume relationship

EDV = end-diastolic volume

E_{es} = elastance end-systole

ESPVR = end-systolic pressure-volume relationship

ESV = end-systolic volume

HF = heart failure

HFrEF = heart failure with reduced ejection fraction

IR = ischemia-reperfusion

LAD = left anterior descending coronary artery

LV = left ventricle/ventricular

LVEDV = left ventricular end-diastolic volume

LVEF = left ventricular ejection fraction

$LVVP_{ed}10$ = left ventricular end-diastolic volume at a pressure of 10 mm Hg

$LVVP_{es}80$ = left ventricular end- systolic volume at a pressure of 80 mm Hg

MI = myocardial infarction

PKA = protein kinase A

PKC = protein kinase C

PLN = phospholamban

PRSW = pre-load recruitable stroke work

RBX = ruboxistaurin

SUMMARY

Inotropic support is often required to stabilize the hemodynamics of patients with acute decompensated heart failure; while efficacious, it has a history of leading to lethal arrhythmias and/or exacerbating contractile and energetic insufficiencies. Novel therapeutics that can improve contractility independent of beta-adrenergic and protein kinase A-regulated signaling, should be therapeutically beneficial. This study demonstrates that acute protein kinase C-α/β inhibition, with ruboxistaurin at 3 months' post-myocardial infarction, significantly increases contractility and reduces the end-diastolic/end-systolic volumes, documenting beneficial remodeling. These data suggest that ruboxistaurin represents a potential novel therapeutic for heart failure patients, as a moderate inotrope or therapeutic, which leads to beneficial ventricular remodeling. (J Am Coll Cardiol Basic Trans Science 2017;2:669–83)

Cardiac pump function is reduced in heart failure (HF) secondary to acute myocardial infarction (MI) and other diseases that cause ventricular dilation. In addition to reductions in basal function, there is a lack in contractile reserve (1). This syndrome is termed HF with reduced ejection fraction (HFrEF). Inotropic therapy is often required to improve pump function and support central hemodynamics in acute decompensated HF (ADHF) (2). However, most inotropic agents currently in clinical practice activate protein kinase A (PKA) or alter key downstream inotropic mediators (3). PKA signaling is the principle mechanism to regulate cardiac contractility in the normal heart. Persistent activation of PKA signaling is required in the failing heart to maintain central hemodynamics, and this leads to disruption of the signaling cascade and blunted adrenergic responsiveness. Drugs such as milrinone increase cyclic adenylate monophosphate (cAMP) by inhibiting the phosphodiesterase (PDE III in this case) that catalyze cAMP inactivation and thereby increase PKA activation. These drugs have reduced efficacy when compared with the normal heart, but are still potent inotropes that can improve cardiac function in patients who present with ADHF (4). However, these drugs can induce lethal arrhythmias (5), and because they can also cause Ca^{2+} overload in the sarcoplasmic reticulum (SR), they can induce myocyte death (6) and thereby exacerbate HF progression. Therefore, patients that survive episodes of ADHF that required the use of PKA-activating inotropic support, have a worse prognosis (7). Novel inotropic therapies that increase cardiac contractility and have positive effects on ventricular remodeling, but avoiding PKA signaling, could help patients with HF.

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Over the last decade, we (8–12) and others (13–17) have investigated the role of protein kinase C (PKC) in the alterations of basal inotropy and inotropic reserve in small-animal HF models. PKC is a family of serine/threonine protein kinases, which are activated through Ca^{2+}- and/or lipid-mediated signaling mechanisms, and are enhanced in HF. The major PKC isoform expressed in cardiac tissue of small (mouse) (18) and large (rabbit) (19) animals, as well as in humans, is PKCα (12). PKCα abundance and activity increases in the diseased heart and has been linked to reduced cardiac myocyte contractility, whereas PKCα inhibition increases cardiac contractility (10). PKCα-target proteins are distinct from those activated by PKA (4,20) and include classical Ca^{2+} handling and regulator proteins within the membrane, cytosol, and at the level of sarcomeric proteins (10,15–17,21). Studies performed largely in mouse models suggest that inhibition of PKCα could be a PKA-independent approach to increase contractility in the failing human heart (9,10). However, regulation of cardiac contractility is fundamentally different in rodents and large mammals, including humans, and this may explain why so many Ca^{2+}-dependent therapeutic strategies that have worked in rodent models have not translated to effective therapeutics in humans (22,23). Previously we tested the chronic administration of ruboxistaurin (RBX) in a farm pig model of MI (24). Ladage et al. (24) reported that RBX had beneficial effects on cardiac contractility (dP/dTmax) and remodeling (left ventricular [LV] ejection fraction [LVEF]) at 3 months' post-MI. Although RBX was administered throughout the study (10 mg/kg/day), beneficial effects were only observed at the 3-month time point. This could be due to the normal hypertrophic growth observed in farm pigs over the study timeline or that sufficient cardiac pathology