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EDITORIAL COMMENT

Is Protein Kinase C Inhibition the Tip of the Iceberg in New Therapeutics for Acutely Decompensated Heart Failure?*

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Heart failure with reduced ejection fraction (HFrEF) or systolic heart failure (as it was commonly known) is a disease of impaired contractility. Long-term inhibition of the neurohormonal system is pivotal to the chronic treatment of HFrEF, which allows for reverse remodeling and is currently the mainstay of treatment, resulting in improved morbidity and mortality. However, targeted therapy to improve contractile function in HFrEF has been less successful, and for acutely decompensated heart failure (ADHF), positive inotropic drugs have consistently fallen short of expectations despite showing improved short-term hemodynamic responses in these patients (1). In fact, prolonged use of positive inotropes increases mortality (2). However, management of ADHF continues to weigh heavily on patient outcomes, health providers, and the health care system as a whole, and as such, there has been no approval of a positive inotrope by the U.S. Food and Drug Administration (FDA) in more than 25 years, since milrinone (Primacor) was approved in 1987 and its generic, milrinone, in 2002.

Positive inotropic drugs increase contractility and pump function in HFrEF, either by increasing myosin activity directly (3) or intracellular calcium flux (4), resulting in improved contractile force of the myofilament proteins and, as such, improved cardiac output in ADHF and HFrEF. However, prolonged use of positive inotropic agents worsens outcomes, and some are proarrhythmic, the mechanism of which is thought to be due to increased myocardial oxygen consumption (5) or chronotropic effects through stimulation of β-1 adrenergic receptors (6). Levosimendan, a calcium sensitizer, increases the sensitivity of the heart to calcium but increases cardiac contractility without a rise in intracellular calcium. In 2 separate trials, (7,8), levosimendan compared to placebo was proarrhythmic despite clinical and symptomatic improvement. Cardiac glycosides inhibit sodium-potassium-ATPase (Na+/K+-ATPase), resulting in sodium accumulation which, in turn, increases cellular calcium. This activates the sodium-calcium exchanger resulting in a positive inotropic action; however, cardiac glycosides have a very narrow therapeutic window (9). The myosin activator omecamtiv mecarbil improves short- term cardiac performance (ATOMIC-AHF) (10) but long-term effects on mortality remain unstudied.

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In this issue of *JACC: Basic to Translational Science*, Sharp et al. (11) demonstrated that acute inhibition of a kinase independent of direct calcium load or myosin activation, PKCα/β, improved contractility and systolic performance in a swine model of post-myocardial infarction (MI) and HFrEF.

The protein kinase C (PKC) family was first discovered 40 years ago. It is a family of homologous proteins activated by proteolysis and by diacylglycerol in the presence or absence of calcium. The groups are further

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subdivided into 4 classic (α, β, βII, γ) and 4 novel (δ, ε, θ, η) isotypes, the latter of which can be activated by diacylglycerol alone. A third group, the atypicals (PKCλ/ι and PKCζ), requires neither calcium nor diacylglycerol for activation. Each of the kinases can be found in the same tissue and activated by the same stimuli. Drug development for these kinases has progressed slowly due to the off-target effects of the drugs, the plasticity of each enzyme in multiple signaling cascades, and the importance and ubiquity of each isoform in different cells (12). In addition, the translation of preclinical or animal models to clinical events has been difficult due to the differential expression of these isoforms from mouse to man (13). As such, only a single drug, ruboxistaurin, has progressed to clinical trials. Ruboxistaurin (Arxxant, Eli Lilly, Annapolis, Maryland) was initially developed as a PKCβ inhibitor (14) and showed some promise in preventing the progression of diabetic retinopathy (dosage: 8 to 32 mg/daily) (15). It was later found also to have PKCα inhibitory activity (16).

Sharp et al. (11) expanded the study by Ladage et al. (17), who showed in a swine model that chronic administration of ruboxistaurin immediately after MI attenuated contractile dysfunction with trends to a reduction in infarct size. What is different in the present study is that Sharp et al. (11) used a chronic ischemic HFrEF model and that therapy was instituted 3 months post-MI as a single (acute) 1-time dose of ruboxistaurin 20 mg/kg (~600 mg). This single dose improved contractile function (measured 2 h after oral administration) compared to that in controls and groups treated with dobutamine (2.5 μg/kg/min) (11). The authors suggest that this phenotype of improved contractile function is likely due to a change in a single phosphorylation site of PKC alpha at threonine (Thr) 638 with ruboxistaurin compared to untreated post-MI control hearts (11).

This highly interesting result should be tempered by several caveats. Although the drug's safety was noted in several human clinical trials, doses used for this pre-clinical model were much greater. In fact, the dose used for the preclinical model exceeded that used in patients by almost 40-fold. In addition, heart failure was not studied in the prior clinical trials. It was relegated to diabetic neuropathy, retinopathy, and eye disease. However, a direct safety and clinical applicability study may be on the horizon. A phase I/II trial will attempt to address efficacy and safety with 30 patients with New York Heart Association functional class III or IV HFrEF (NCT02769611) in a dose escalation study (using 3 doses in groups of 10 patients: 64 mg, 128 mg, and 256 mg) as a single acute administration of ruboxistaurin. Change in contractile function, which will be determined by echocardiography, is a secondary outcome, as are symptom relief and 30-day outcomes. Primary outcomes are changes in biomarkers and electrocardiography. Importantly, it should be noted these doses in the proposed phase I/II trial will still be almost 6-fold less than doses used in the present preclinical study by Sharp et al. (11).

Surprisingly, blood pressure went unmeasured in the animals during the study as PKCα inhibition can lead to hypotension in rodents (18), and this change in afterload by itself can also lead to improvements in cardiac output. Last, the comparison to very low doses of dobutamine (2.5 μg/kg/min) (11), which acts primarily on β-1 adrenergic receptors, may have made the effects of ruboxistaurin seem much more pronounced and robust (19). Importantly, in the Survival of Patients With Acute Heart Failure in Need of Intravenous Inotropic Support (SURVIVE) study of levosimendan in humans, the comparison was similar in that there was a dobutamine arm. However, the dobutamine doses began at 5 μg/kg/min with the ability to escalate to 40 μg/kg/min (8). Mortality was greater in levosimendan than in placebo subjects, but there was a nonsignificant trend to improved survival compared to dobutamine subjects (8).

Mechanistically, PKCα and -β can change contractility in rodent models (20,21). Since the epitopes for a PKCα Thr 638 and PKC βII Thr 641 are very similar (22,23), it is possible that there may have been cross-reactivity using the antibody detection method, but it remains unclear what antibodies were used in this present study. Despite this, the identification of a specific phosphorylation site of the protein kinase C that changes contractility would make for an attractive scientific and therapeutic target. It would be interesting to know whether active (Glu) or inactive (Ala) PKC Thr 638 mutation leads to alterations in contractility in vitro or in vivo. In addition, presumptive binding partners of phospho-PKC Thr 638 could be determined to allow for the development of "separation of function inhibitors (peptides that are designed to selectively inhibit the phosphorylation of one particular substrate of an individual PKC isozyme without affecting the phosphorylation of its other substrate" (12). A likely candidate for this would be a myofilament protein like troponin, which PKCα has been shown to phosphorylate (24).

Last, the suggestion that a single dose of ruboxistaurin purportedly reduces "heart size" should be treated with restraint. There is a reduction in left ventricular volume, as shown by echocardiography and invasive hemodynamics, but no evidence was

shown that ruboxistaurin decreases cardiac hypertrophy. A reduction in afterload by lowering blood pressure may affect the volume of the ventricle. Furthermore, it is unknown whether the reduction in ventricular volume is a persistent finding, and neither is the mechanism whereby the ventricular volume is altered is addressed further in the study. We have learned from human studies with ADHF that even 24 to 48 h of continuous intravenous administration of positive inotropic therapy is unlikely to engender prolonged benefit in ADHF (25).

Since the use of foxglove in the 1700s (26), a drug that may enhance contractility of the heart and improve outcome in HFrEF and ADHF has been the holy grail with a number of unfulfilled promises (1,8). Similarly, drug therapy, using inhibition of PKC, has been littered with failures since the discovery of the enzyme family 40 years ago. The importance of finding a use for a PKC inhibitor in therapy for ADHF and HFrEF represents a first small step. Coupled to an acute improvement in cardiac contractile function as an inotropic agent makes it a very large initial step. The finding that a PKCα/-β inhibitor is capable of acutely increasing cardiac contractility after a single dose in a chronic ischemic HFrEF large-animal study (11) is a laudable first step toward a new therapeutic insight for HFrEF and ADHF. Although it is still early, this study gives hope for drug development in that there are mechanistic insights as to the increased contractility seen in this HFrEF model. Similarly, optimism for therapeutic options is in sight as we chip away at the tip of the iceberg for therapies for ADHF patients, a group with a very large unmet clinical need.

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