JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 1, NO. 7, 2016

ISSN 2452-302X
http://dx.doi.org/10.1016/j.jacbts.2016.10.005

EDITORIAL COMMENT

Fine Tuning Adenylyl Cyclase as a (Gene) Therapy for Heart Failure*

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Heart failure is an increasingly prevalent disease that imposes significant clinical and economic burdens on modern societies (1). Standard pharmacological treatments of heart failure with reduced left ventricular ejection fraction (HFrEF), includes beta-adrenergic receptor (β-AR) blockers, inhibitors of the renin-angiotensin-aldosterone system (RAAS), and diuretic agents. Beta-blockers and RAAS inhibitors improve survival and quality of life, but typically do not normalize either (1). Recently, 2 new small-molecule therapeutics, sacubitril/valsartan (2) and ivabradine (3), have been added to the HFrEF armamentarium, but the effects of either are incremental and much remains to be done before HFrEF therapy can be considered an unqualified success. In addition, the other major type of heart failure, heart failure with preserved left ventricular ejection fraction, lacks any therapy demonstrated to improve natural history. These limitations have naturally motivated the development of alternative therapeutic approaches beyond small-molecule pharmacologics, including device therapies, stem cell biologics, and genetically-based therapies (including pharmacogenetic approaches and gene therapy).

Relevant to gene therapy of heart failure, in the current issue of *JACC: Basic to Translational Science*, Gao et al. (4) demonstrate in a mouse model that cardiac-directed expression of a catalytic domain (C1C2) construct of adenylyl cyclase 6 (AC6) can reverse cardiac dysfunction produced secondary to sustained β-AR stimulation with isoproterenol. The concept of using gene therapy expression of AC, namely AC6, has been a pursuit of Hammond et al. (5) for more than a decade (5), and has transitioned fully from preclinical work to a recently completed phase 2 clinical trial (6). This trial demonstrated safety of the treatment and a dose-range possibly associated with an efficacy signal.

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It is now generally accepted that chronic stimulation of the β-AR/Gαs/AC/PKA/CaMKII signaling pathway, as occurs in heart failure via sustained cardiac adrenergic stimulation, can be deleterious to a damaged heart (7). The clinical correlate to this finding is that inhibition of the β-AR signaling pathway by beta-blockers, in conjunction with RAAS inhibition, is the current gold standard of therapy in patients with HFrEF (1,8).

A number of studies have dissected the canonical β-AR signaling pathway at multiple levels, demonstrating that overexpression of β_1-AR (9), Gαs (10), PKA (11), and CaMKII (12) can all have deleterious effects. The lone exception to these signaling pathway components is AC, where overexpression of the calcium-inhibitable isoforms is of apparent benefit to compromised cardiac function (13,14). Reciprocally, deletion of AC6 (knock out mice) in the face of sustained β-AR stimulation appears to increase mortality (15). Interestingly, the positive attributes of AC overexpression occur even with a catalytically inactive construct (13), supporting the notion that the salutary effects of AC6 are independent of PKA-mediated phosphorylation. The question, therefore, is: what makes AC unique among members of the β-AR effector pathway? The answer almost certainly

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

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lies in the fact that AC stimulation can have effects that are permissive of enhanced calcium handling independent of cyclic adenosine monophosphate (cAMP) generation (4,13,16).

In the current study, Hammond et al. (4) demonstrates in transgenic mice that cardiac-restricted expression of the cytoplasmic domains of AC6 are associated with normal LV structure and function. More importantly, transgenic C1C2 expression appears to partially abrogate the ventricular dysfunction resulting from subacute β-AR stimulation with isoproterenol. Whereas isoproterenol decreased both positive and negative dP/dt in control animals, C1C2-expressing animals demonstrated an increase in both parameters after a 7-day infusion. The fact that C1C2 expression also resulted in increased isoproterenol-mediated Ca^{2+} release and increased SERCA2a expression appears to be the likely explanation for these salutary effects. In addition, in isolated neonatal rat cardiac myocytes subjected to direct adenovirus gene transfer with the C1C2 construct, there was evidence of inhibition of β-AR-mediated generation of cAMP, as well as increased phosphorylation of Akt and its downstream targets. The downstream Akt effects are shared with catalytically active AC6 (17), but inhibition of β-AR signaling to endogenous membrane-bound ACs resulting in decreased cAMP generation is unique to the C1C2 construct. Thus, C1C2 appears to function as an inhibitor of β-AR/cAMP signaling, which may explain some of the observed functional and biochemical effects in the isoproterenol infusion mice. In human HFrEF, long-term treatment with beta-blockers also increases positive dP/dt (18) and SERCA2a expression (19). However, in contrast to the C1C2 transgenic mice infused with isoproterenol, beta-blockers in human HFrEF substantially increase left ventricular ejection fraction (18,19) and do not increase afterload, which is the apparent explanation for the lack of improvement in fractional shortening in the isoproterenol-infused C1C2 overexpression transgenic mice (4).

Whether or not the C1C2 construct would fare better in gene therapy protocols than the original full-length AC6 will have to be determined in future clinical trials. In this regard, gene therapy for heart failure is in its infancy and faces major challenges including adequate uptake of the vector-construct into cardiac myocytes, a high cost of goods, the requirement for invasive procedures for delivery, and possible reduction in gene dose by pre-formed antibodies to the vector. Nevertheless, some phase 2 trials of heart failure gene therapy have been promising (6,20), and the scope of the heart failure problem coupled with the limitations of current therapy justify continued efforts to develop more ideal gene targets as well as improved delivery to the failing heart.

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KEY WORDS adenylyl cyclase, gene therapy, heart failure