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Gene Therapy With Angiotensin-(1-9) Preserves Left Ventricular Systolic Function After Myocardial Infarction

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ABSTRACT

BACKGROUND Angiotensin-(1-9) [Ang-(1-9)] is a novel peptide of the counter-regulatory axis of the renin-angiotensin-aldosterone system previously demonstrated to have therapeutic potential in hypertensive cardiomyopathy when administered via osmotic mini-pump. Here, we investigate whether gene transfer of Ang-(1-9) is cardioprotective in a murine model of myocardial infarction (MI).

OBJECTIVES The authors evaluated effects of Ang-(1-9) gene therapy on myocardial structural and functional remodeling post-infarction.

METHODS C57BL/6 mice underwent permanent left anterior descending coronary artery ligation and cardiac function was assessed using echocardiography for 8 weeks followed by a terminal measurement of left ventricular pressure volume loops. Ang-(1-9) was delivered by adeno-associated viral vector via single tail vein injection immediately following induction of MI. Direct effects of Ang-(1-9) on cardiomyocyte excitation/contraction coupling and cardiac contraction were evaluated in isolated mouse and human cardiomyocytes and in an ex vivo Langendorff-perfused whole-heart model.

RESULTS Gene delivery of Ang-(1-9) reduced sudden cardiac death post-MI. Pressure volume measurements revealed complete restoration of end-systolic pressure, ejection fraction, end-systolic volume, and the end-diastolic pressure volume relationship by Ang-(1-9) treatment. Stroke volume and cardiac output were significantly increased versus sham. Histological analysis revealed only mild effects on cardiac hypertrophy and fibrosis, but a significant increase in scar thickness. Direct assessment of Ang-(1-9) on isolated cardiomyocytes demonstrated a positive inotropic effect via increasing calcium transient amplitude and contractility. Ang-(1-9) increased contraction in the Langendorff model through a protein kinase A–dependent mechanism.

CONCLUSIONS Our novel findings showed that Ang-(1-9) gene therapy preserved left ventricular systolic function post-MI, restoring cardiac function. Furthermore, Ang-(1-9) directly affected cardiomyocyte calcium handling through a protein kinase A–dependent mechanism. These data emphasized Ang-(1-9) gene therapy as a potential new strategy in the context of MI. (J Am Coll Cardiol 2016;68:2652–66) © 2016 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

The renin-angiotensin-aldosterone system (RAAS) maintains cardiovascular homeostasis through angiotensin II (Ang II). Clinically, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers are mainstay treatments for hypertension and heart failure (HF). Following myocardial infarction (MI), RAAS inhibition stabilizes adverse cardiac remodeling and function and limits progression to HF.

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A natural counter-regulatory axis of the RAAS exists, centered on ACE2, an ACE homologue that metabolizes Ang II to angiotensin-(1-7) [Ang-(1-7)] (1,2). Currently being explored therapeutically in cardiovascular diseases including HF and pulmonary hypertension, ACE2 shows promising therapeutic effects (3). Ang-(1-7) acts via the receptor Mas to block detrimental effects of Ang II and mediates direct therapeutic effects in cardiovascular disease (4,5). Ang-(1-7) is in clinical trials to treat diabetic foot ulcers and cancer (6,7), emphasizing translational approaches targeting the counter-regulatory RAAS axis.

Less studied than Ang-(1-7), the alternative counter-regulatory RAAS peptide angiotensin-(1-9) [Ang-(1-9)] reduces adverse cardiovascular remodeling in rat models of hypertension and MI following peptide administration via osmotic mini-pump (8–10). Ang-(1-9) attenuates cardiomyocyte hypertrophy and cardiac fibrosis in hypertensive models; these effects are blocked by coadministration of the angiotensin type 2 receptor (AT_2R) antagonist PD123,319, further supporting independent effects of Ang-(1-9) as a new counter-regulatory RAAS axis peptide (8,11).

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Assessment of RAAS peptides as therapeutics is limited by short circulatory half-life, requiring osmotic mini-pumps for sustained release in vivo in experimental models. Accordingly, alternative delivery strategies are required for clinical translation. Viral gene therapy is being pursued for HF, including clinical trials using adeno-associated virus (AAV) vector-mediated delivery of sarcoplasmic endoreticulum calcium adenosine triphosphatase 2a (SERCA2a), emphasizing safety and clinical utility (12).

Angiotensin peptides are not produced from genes, but are generated extracellularly in the circulation. Synthetic expression cassettes for Ang II, Ang-(1-7), and Ang-(1-9) have been utilized in transgenic models and in gene transfer approaches (13–15). Here, for the first time, in vivo AAV-mediated gene transfer of Ang-(1-9) via a synthetic expression cassette has been utilized to study cardiac effects in a murine model of MI.

METHODS

Detailed methods are presented in the Online Appendix. Briefly, an Ang-(1-9) expression cassette (13) was sub-cloned into plasmid adeno-associated virus-multiple cloning site (pAAV-MCS) and AAV9 vectors produced via standard protocols (16). Surgical procedures were performed in accordance with the Animals Scientific Procedures Act (1986) and approved by the University of Glasgow Animal Welfare and Ethical Review Panel and UK Home Office. For MI, the left anterior descending artery (LAD) was ligated. Sham animals had identical procedures without ligation. AAVAng-(1-9) or AAV green fluorescent protein (GFP) were delivered intravenously via tail vein following MI as described (17). Echocardiography was performed weekly (**Figure 1A**) and pressure volume (PV) loop measurements made. Fibrosis was assessed by Picrosirius red staining as described (8). Hypertrophy was measured by wheat germ agglutinin staining. Quantitative reverse transcription polymerase chain reaction was assessed with inventoried gene expression assays. Ventricular cardiomyocytes were isolated from adult C57BL/6 mice, loaded with Fura-4FAM, and the Fura-4FAM fluorescence ratio (340/380 nm excitation) was measured using a spinning wheel monochromator and converted to $[Ca^{2+}]_i$ (18). Cardiomyocytes were incubated for 15 min with 1 μmol/l Ang-(1-9), field-stimulated (1.0 Hz), and perfused with 1.8 mmol/l $[Ca^{2+}]_o$ HEPES superfusate containing 1 μmol/l Ang-(1-9). Calcium transients and contractility in human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CM; iCell^2 cardiomyocytes, Cellular Dynamics International [Madison, Wisconsin, USA]) were measured in the optical platform CellOPTIQ (Clyde Biosciences Ltd, Glasgow, United Kingdom) in cells loaded with 3 μmol/l Fura-4F-AM. Calcium transients were obtained from the 360/380 ratio and contraction was assessed using a high-resolution camera coupled to CellOPTIQ. Male adult Wistar rats were sacrificed, hearts excised, and Langendorff perfused at 37°C and constant flow (10 ml/min) (19). A fluid-filled balloon was inserted into the left ventricle and connected to a solid-state pressure transducer. Hearts were paced and perfused with 1 μmol/l Ang-(1-9).

STATISTICAL ANALYSIS. Data are represented as mean ± SE of the mean (SEM). Paired Student *t* test for direct comparisons and 1-way analysis of variance with Tukey's post-test for multiple comparison were performed. Echocardiography was analyzed using repeated measures analysis of variance with Tukey's post-test. Statistical significance was demonstrated with a $p < 0.05$.

RESULTS

Previously, tail vein delivery of 1×10^{11} viral genomes (vg) AAV9 demonstrated robust cardiac transduction (17). To confirm this, AAVGFP-mediated transduction was assessed at 1, 2, and 8 weeks

ABBREVIATIONS AND ACRONYMS

Ang = angiotensin

AT_2R = angiotensin type 2 receptor

CO = cardiac output

FS = fractional shortening

LV = left ventricular

MI = myocardial infarction

RAAS = renin-angiotensin-aldosterone system

SERCA = sarcoplasmic endoreticulum calcium adenosine triphosphatase