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GYY4137 attenuates remodeling, preserves cardiac function and modulates the natriuretic peptide response to ischemia

Shera Lilyanna [a,b], Meng Teng Peh [c], Oi Wah Liew [a,b], Peipei Wang [a,b], Philip K. Moore [c], Arthur Mark Richards [a,b,d,e,1], Eliana C. Martinez [a,b,f,*,1]

[a] Cardiovascular Research Institute, National University Health System, Singapore
[b] Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[c] Neurobiology Group, Life Sciences Institute and Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[d] Cardiac Department, National University Health System, Singapore
[e] Christchurch Heart Institute, University of Otago, Christchurch, New Zealand
[f] Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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ABSTRACT

Aims: Myocardial infarction followed by adverse left ventricular (LV) remodeling is the most frequent proximate cause of heart failure. Hydrogen sulfide (H_2S) is an important endogenous modulator of diverse physiological and pathophysiological processes. Its role in post-ischemic ventricular remodeling and the associated neurohormonal responses has not been defined. Here, we aimed at evaluating whether the slow-releasing water-soluble H_2S donor GYY4137 (GYY) exerts cardioprotective effects and modulates the neurohormonal response to cardiac ischemic injury.

Methods and results: Treatment for 2 or 7 days with GYY (100 mg/Kg/48 h, IP) after acute myocardial infarction (MI) in rats preserved LV dimensions and function in vivo, compared to untreated infarcted (MI), placebo- and DL-propargylglycine- (PAG, an inhibitor of endogenous H_2S synthesis) treated animals (n = 9/group/time-point). LV dimensions and function in GYY-treated animals were comparable to healthy sham-operated rats. GYY-treated hearts had significantly less LV fibrosis than MI, placebo and PAG hearts. A higher density of blood vessels was found in the LV scar area of GYY-treated animals compared to all other infarcted groups. Despite preserved LV structure and function, treatment with GYY increased the levels of the natriuretic peptides ANP and BNP in association with enhanced cyclic GMP levels, paralleled by higher cGMP-dependent protein kinase type I (cGKI) protein levels.

Conclusions: Our data suggest that the slow-releasing H_2S donor, GYY4137, preserves cardiac function, attenuates adverse remodeling and may exert post-ischemic cardioprotective (pro-angiogenic, anti-apoptotic, anti-hypertrophic and anti-fibrotic) effects in part through enhanced early post-ischemic endogenous natriuretic peptide activation.

1. Introduction

Myocardial infarction (MI) complicated by adverse left ventricular (LV) remodeling is the most frequent proximate cause of heart failure (HF), itself a leading cause of death world-wide [1,2]. Despite advances in therapy, this mandates a continued search for new therapeutic strategies targeting the deleterious endogenous responses following MI. Neurohormonal activation, particularly of renin–angiotensin–aldosterone and sympathetic nervous systems, occurs after MI promoting adverse ventricular remodeling which may culminate in HF [3]. The natriuretic peptide (NP) family of endogenous hormones plays important roles in the regulation of cardiovascular and renal functions. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are released by the heart into the circulation normally not only in response to myocardial stretch but also in response to ischemia [4]. Activation

Abbreviations: ANP, atrial natriuretic peptide; BCL-2, B-cell CLL/lymphoma 2; BNP, B-type natriuretic peptide; cGKIα, guanosine 3′5′-cyclic monophosphate-dependent kinase I alpha; cGMP, guanosine 3′5′-cyclic monophosphate; CO, cardiac output; CSE, cystathionine γ-lyase; EF, ejection fraction; eNOS, endothelial nitric oxide synthase; GYY4137, morpholin-4-ium-4-methoxyphenyl (morpholino)phosphinodithioate salt; FS, fractional shortening; H_2S, hydrogen sulfide; IP, intra-peritoneal; LAD, left anterior descending coronary artery; LV, left ventricular; LVAWd, left ventricular anterior wall in diastole; LVAWs, left ventricular anterior wall in systole; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; MI, myocardial infarction; NaHS, sodium hydrosulfide; NPs, natriuretic peptides; NPR1, natriuretic peptide receptor 1; NPR3, natriuretic peptide receptor 3; PAG, DL-propargylglycine; p-eNOS, phospho-endothelial nitric oxide synthase; PRA, plasma renin activity; SC, subcutaneous; VEGF-A, vascular endothelial growth factor-A.

* Corresponding author at: Department of Pediatrics, Division of Cardiology, University of Miami Miller School of Medicine, 1501 N.W., 10th Avenue, Room 803, Miami, FL 33136, United States.
E-mail address: ecm37@med.miami.edu (E.C. Martinez).
[1] Both authors contributed equally as senior authors.

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of the NP receptor-1 (NPR-1), the receptor for ANP and BNP, increases its second messenger cyclic guanosine monophosphate (cGMP), which mediates natriuresis and inhibition of cardiac sympathetic nerve traffic, renin and aldosterone release, and displays vasorelaxant, anti-fibrotic, anti-hypertrophic, antioxidant and pro-angiogenic effects [5,6]. Compelling experimental and clinical data support a beneficial compensatory role for endogenous NPs in acute and chronic cardiac injury, and exogenous recombinant NPs are used therapeutically in clinical MI and acute HF, despite mixed results from therapeutic trials [7–11].

Hydrogen sulfide (H_2S) is emerging as an important endogenous biogas that exhibits cytoprotective effects in diverse physiological and pathophysiological settings [12]. It preserves cell function and protects against numerous injurious stimuli through activation of signaling pathways that are protective in cardiac injury [13–15]. In vitro, high concentrations of H_2S inhibit angiotensin-converting enzyme (ACE) in endothelial cells [16]. However, the possible influence of H_2S on neurohormonal activation after cardiac ischemic injury is unknown. A slow-releasing H_2S donor, morpholin-4-ium-4-methoxyphenyl (morpholino) phosphinodithioate (GYY4137), has recently been introduced, and its antihypertensive [17], anti-atherosclerotic [18], anti-inflammatory, [15] and anti-cancer [19] activities in vitro and in vivo have been described. The effects of GYY4137 on LV remodeling and function following myocardial injury have not been characterized. We tested the hypothesis that GYY4137 is cardioprotective in a rat model of myocardial infarction. The neurohormonal response is pivotal in the evolution from MI to adverse LV remodeling; all pharmacotherapies currently proven to ameliorate adverse remodeling in clinical HF operate via neurohormonal pathways [20] and therefore any benefit from an experimental agent may be reflected in, and possibly mediated through, effects upon neurohormonal effectors. Hence, we also hypothesized that GYY4137 may exert cardioprotective effects in part through modulation of the neurohormonal response to cardiac injury.

2. Methods

2.1. Rat model of myocardial infarction and therapeutic intervention

All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore (Protocol No 064/11). Male Wistar Rats (250–300 g) underwent left anterior descending coronary artery (LAD) ligation as previously described [21] Treatment was initiated immediately (30 min) after coronary ligation as follows: untreated myocardial infarction (MI), placebo (0.9% saline solution, 1.5 mL/24 h, IP), GYY (GYY4137, 100 mg/kg/48 h, IP) and PAG (DL-propargylglycine, an inhibitor of cystathionine-γ lyase, 50 mg/kg/24 h, IP; Sigma Aldrich, MO, USA). Controls were sham thoracotomy-operated rats. Transthoracic echocardiography was performed in all animals at baseline (before surgery) and at end-point (i.e. 2 and 7 days after MI) using a Vivid 7 Dimension ultrasound system equipped with a broadband 10S transducer (GE VingMed Horton, Norway) as previously described [21]. LV pressure and volume measurements were performed at end-point using a pressure transducer catheter and a 2-mm transient-time flow probe positioned around the ascending aorta [21]. Blood was retrieved by cardiac puncture into EDTA tubes and heart tissue was processed for histology, mRNA and protein extraction. Plasma was stored at −80 °C until required and then thawed and assayed as described in the Online Supplemental Methods.

2.2. Statistical analysis

Data are presented as mean ± SEM. Inter-group comparisons of echocardiographic indices were by 2-way ANOVA with repeated measures followed by pair-wise comparisons by Bonferroni's post-test. The ANOVA model included control versus treatment and baseline versus 2 or 7 days after MI as factors, as well as the interaction between the two factors. For other comparisons, one-way ANOVA followed by Bonferroni's-post-hoc test and unpaired Student's t-test were used when appropriate. Differences were considered significant when $P < 0.05$. All statistical analyses were performed using GraphPad Prism® software version 5.04 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Animal survival

All the rats from the sham-operated group survived the surgical procedure (n = 6/time-point). In total, 12 rats out of 84 that underwent LAD ligation died within 24 h after surgery as follows: 3 out of 21 MI (14.3%), 3 out of 21 placebo (14.3%), 2 out of 20 GYY (10%) and 4 out of 22 PAG (18.2%) groups. Thus, 9 infarcted rats/group/time-point were included in this study.

3.2. Effect of GYY4137 on the H_2S system during ischemic injury

Plasma H_2S levels, measured by high-performance liquid chromatography (HPLC), were increased 2.8 fold by GYY4137 compared to sham rats ($P < 0.0001$) (Fig. 1A). Plasma H_2S levels on days 2 and 7 in GYY-treated animals were 2.3 ± 0.2 and 3.1 ± 0.4 μmol/L respectively, and significantly enhanced at both time-points compared to untreated MI (1.2 ± 0.1) and (1.5 ± 0.1 μmol/L; $P < 0.01$ and $P < 0.001$), placebo- (1.1 ± 0.1 and 1.7 ± 0.2 μmol/L; $P < 0.001$ and $P < 0.01$) and PAG-treated rats (1.4 ± 0.1 and 1.6 ± 0.2 μmol/L; $P < 0.01$, respectively). Protein expression of cystathionine-γ lyase (CSE) in liver tissue was not altered (Supplemental Fig. S1A and S1C). The effect of PAG on tissue H_2S synthesizing enzyme activity was assessed in liver homogenates from PAG-treated animals which produced 31-fold less H_2S (nmol/mg of soluble protein) at day 2 (0.20 ± 0.01) and day 7 post-MI (0.14 ± 0.01) compared to placebo-treated animals (5.97 ± 0.37, and 4.38 ± 0.31; $P < 0.0001$ for both) (Supplemental Fig. S2D).

3.3. Treatment with GYY4137 preserves cardiac function and structure following myocardial infarction

Echocardiographic assessment of LV remodeling and function at 2 and 7 days after MI revealed that GYY4137 preserved cardiac function and attenuated post-MI remodeling (Fig. 1B and Online Supplement Tables S2 and S3). Administration of GYY4137 after MI at 48 hr intervals over a week attenuated early adverse cardiac remodeling with preservation of LV internal dimension in systole (LVIDs) and diastole (LVIDd) in GYY-treated rats compared to untreated infarcted (MI), placebo and PAG-treated animals at both 2 and 7 days post-MI. LV dimensions in systole and diastole in GYY and sham animals were comparable. While all other infarcted groups exhibited a significant increase in LVIDs at day 2 ($P < 0.0001$), GYY-treated rats did not. LVIDd and LVIDs increased in GYY-treated rats from baseline to day 7 after MI ($P < 0.01$ and $P < 0.001$, respectively), but this was ameliorated in comparison with MI, placebo- and PAG-treated animals ($P < 0.0001$ for all comparisons). Therapeutic intervention with GYY led to the preservation of wall thickness and thickening at day 2, with values in systole comparable to baseline and to sham animals, whereas MI, placebo and PAG hearts underwent a significant decrease in LV wall thickness compared to baseline and GYY-treated hearts (Fig. 1B and Supplement Table S2). Evaluation at day 7 post-MI showed that LV wall thickness in GYY-treated rats was reduced compared to baseline ($P < 0.05$) but comparable to sham, while the other infarcted groups exhibited a marked decrease in wall thickness compared to sham and to baseline ($P < 0.0001$) (Fig. 1B and Supplement Table S3). GYY-treated animals had thicker LV anterior walls than MI ($P < 0.01$) and PAG-treated animals ($P < 0.05$) at day 7. LVESV, a cardinal post-MI predictor of adverse remodeling and increased risk of adverse clinical outcomes [22], was unchanged in rats treated with GYY and it was