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ORIGINAL RESEARCH ARTICLE

Nitrates in combination with hydralazine in cardiorenal syndrome: a randomized controlled proof-of-concept study

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Abstract

Aims Cardiorenal syndrome (CRS) is a common problem of great morbidity and mortality. Hydralazine–isosorbide dinitrate (H-ISDN) may be used in renal failure and may improve exercise capacity in heart failure (HF). Our proof-of-concept study aimed to evaluate early evidence of efficacy, safety, and feasibility of H-ISDN compared with standard of care in CRS.

Methods and results This multi-centre, single-blind, randomized trial in Singapore enrolled CRS patients, defined as chronic HF with concomitant renal failure [estimated glomerular filtration rate (eGFR) $<$ 60 ml/min/1.73 m^2]. The primary outcome was 6 min walk test (6MWT) distance measured at 6 months. Secondary outcomes included study feasibility; efficacy outcomes which included renal, cardiac, and endothelial functions, health-related quality of life using Short Form-36, clinical outcomes; and adverse events. Forty-four patients [71 ± 10 years; 75% male; median (inter-quartile range) N-terminal prohormone brain natriuretic peptide 1346 (481–2272) pg/mL] with CRS (left ventricular ejection fraction 42 ± 12% and eGFR 46 ± 15 ml/min/1.73 m^2) were randomized into two equal groups. Of these, 39 (89%) had hypertension, 27 (61%) had diabetes mellitus, and 17 (39%) had atrial fibrillation. Six (27%) discontinued H-ISDN owing to intolerance and poor compliance. There was a trend towards improved 6MWT distance with H-ISDN compared with standard of care at 6 months (mean difference 27 m; 95% CI, −12 to 66), with little differences in secondary efficacy outcomes. Giddiness and hypotension occurred more frequently with H-ISDN, but HF hospitalizations and mortality were less.

Conclusions Our pilot study does not support the addition of H-ISDN on top of standard medical therapy to improve exercise capacity in patients with CRS.

Keywords Cardiorenal syndrome; Hydralazine; Isosorbide dinitrate; Exercise capacity; Endothelial function

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This study was conducted in National University Hospital, National Heart Center, and Changi General Hospital.
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Introduction

Cardiorenal syndrome (CRS), where heart failure (HF) and renal failure co-exist in a vicious cycle, is a very common problem of great morbidity and mortality. Data from the prospective ASIAN-HF registry showed a high prevalence of chronic kidney disease (CKD) amongst chronic HF patients in Asia, with Southeast Asia reporting the highest prevalence at 54%,[1] in whom CKD was independently associated with higher 1 year mortality. The management of CRS is

challenging, as therapeutic options may be mutually contradictory. Clinical HF trials have largely excluded populations with severe renal dysfunction [estimated glomerular filtration rate (eGFR) $<$ 30 mL/min], leading to a lack of evidence-based therapies in these patients.[2] Furthermore, the pathophysiology underlying CRS has not been completely elucidated.

Endothelial dysfunction, consequent to reduced bioavailability of nitric oxide (NO), has recently emerged as a common link between the failing heart and kidneys in CRS.[3] Exogenous NO donors such as isosorbide dinitrate (ISDN) increases NO bioavailability, while concomitant hydralazine (H) therapy prevents nitrate tolerance and protects NO from oxidative stress-induced degradation. The synergistic combination of H-ISDN was shown to improve survival and hospitalization risk amongst African-American patients with HF with reduced ejection fraction (HFrEF) in the landmark A-HEFT trial.[4] Its role in non-African-American patients is less clear, though the Vasodilator Heart Failure Trials (V-HEFT) I and II showed improved ejection fraction and exercise tolerance with this combination in white people.[5,6] Guidelines from both the American College of Cardiology/American Heart Association and the Heart Failure Society of America included H-ISDN as a class I recommendation for African-Americans with HFrEF if they were receiving optimal medical therapy and a class IIa recommendation for all races who experience intolerance to angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers.[7] In contrast, nitrates in isolation or combination with hydralazine have not been shown benefit in patients with HF with preserved ejection fraction (HFpEF).[8–10] Furthermore, H-ISDN combination has never been tested in Asian patients on contemporary HF therapy, regardless of EF; neither has it been tested in patients with CRS. There is a high prevalence of endothelial dysfunction in our Asian HF patients, with a greater degree of dysfunction seen in those with CRS (unpublished data). Targeting endothelial dysfunction may improve clinical outcomes amongst Asian patients with CRS.

We performed a prospective randomized controlled proof-of-concept study to assess the effect of H-ISDN therapy for 6 months in Asian patients with CRS. We hypothesized that H-ISDN therapy would lead to an improvement in exercise capacity, with additional benefits in endothelial function, renal function, cardiac structure and function, clinical outcomes, and health-related quality of life (HRQOL).

Methods

Study design

Nitrates In Combination with Hydralazine in cardiorEnal syndrome (NICHE) was an investigator-initiated, randomized, multi-centre, single-blind clinical trial conducted in Singapore. This trial was registered at ClinicalTrials.gov (NCT02343393). Whereas the subjects and investigators were aware of the allocated arm, the outcome assessor was blinded to the treatment allocation.

Subjects were randomized to receive either H-ISDN or standard medical therapy in a 1:1 ratio via permuted block randomization with stratification by centre. The allocation sequence was generated by an independent third party and enclosed in sequentially numbered, opaque, sealed envelopes. The randomization code was kept by the study's main research coordinator. Study investigators performed the randomization.

Study population

Briefly, ambulatory Asian patients with a diagnosis of HF were eligible if they were at least 21 years of age and had concomitant renal dysfunction (eGFR $<$ 60 mL/min/1.73 m^2) and at least one HF admission during the preceding year. Additionally, patients were required to be stable on optimal medical therapy, able to complete 6 min walk test (6MWT), and able to maintain a systolic blood pressure (BP) of at least 100 mmHg.

Exclusion criteria included known hypersensitivity to H-ISDN, concurrent use of phosphodiesterase type 5 (PDE5) inhibitors, rapidly deteriorating HF, or end-stage renal failure (eGFR$<$ 15 mL/min/1.73 m^2, or on regular dialysis, or planned dialysis within the study period). Patients were also excluded if they suffered a cardiovascular event or received cardiovascular interventions within the last 3 months or deemed to require cardiovascular interventions during the study period. The full entry criteria are provided in *Table S1*. All subjects provided written consent.

Study oversight

The study protocol was approved by the local ethics institutional regulatory board and conducted in accordance with the principles of the World Medical Association Declaration of Helsinki on ethics in medical research and International Conference on Harmonization guidelines for Good Clinical Practice. The study sites were responsible for study implementation and clinical management. The Singapore Clinical Research Institute, an independent body, carried out on-site monitoring, data management, and review of safety data throughout the study period.

Study assessment schedule

Clinical assessment, electrocardiogram, blood and urine sampling, 6MWT, and HRQOL assessment were performed after

randomization, prior to administration of treatment. The date of randomization was taken as the baseline. Subjects randomized to treatment arm were initiated on a starting dose of hydralazine 60 mg and ISDN 30 mg daily (20/10 mg three times daily), approximately half of what was used in prior HFrEF studies.[4–6]

All study subjects received a phone call from the study coordinator on Day 7. Apart from evaluating the general condition of subjects, this allowed for the assessment of tolerability of study medication and titration of doses for subjects in treatment arm. They were instructed to double the dose of study medication to the target maintenance dose of hydralazine 120 mg and ISDN 60 mg (40/20 mg three times daily) daily if the starting dose was well tolerated. Study treatment was discontinued in subjects with unacceptable side effects.

Telehealth BP monitoring was conducted for the first month. All subjects were issued with an automated BP monitor during the baseline visit and were instructed to carry out daily home BP monitoring. Particularly for those allocated to intervention arm, this ensured that there was no undetected hypotension (defined as systolic BP $<$ 100 mmHg) after commencing the study drug.

Study subjects were reviewed in the clinic at Weeks 6 and 12, for assessment of clinical stability, adverse reactions to study drug, and compliance to treatment. Compliance to treatment was assessed by pill count; subjects were considered compliant to H-ISDN therapy if the returned pills were $<$20% of what was prescribed. Clinical assessment and evaluation of endpoints were repeated after 6 months. Subjects were followed up remotely for another 2 weeks after the final visit for adverse events.

Study endpoints

The primary endpoint was the mean change in 6MWT at 6 months from the baseline. Assessment was performed by appropriately qualified personnel who were blinded to the treatment allocation of the patients. Every effort was made to have the same member of the site supervise 6MWT for a particular subject. The test was performed in accordance with the American Thoracic Society Guidelines; the distance walked in 6 min, to the nearest metre, was recorded.[11]

Secondary endpoints included change in endothelial function, renal function, cardiac structure and function, clinical outcomes, and HRQOL over 6 months.

Endothelial function was assessed by the non-invasive peripheral arterial tonometry (PAT) using the EndoPAT device (Itamar Medical, Caesarea, Israel) and circulating biomarkers.[12] PAT was conducted in a quiet, temperature-controlled room with the participants lying supine and arms positioned at the level of the heart, following standardized pre-test preparations. PAT probes were placed on both index fingers, with one acting as the 'study' finger and the other as control. After the baseline recording, arterial flow in the 'study' arm was occluded for 5 min using a rapid cuff inflation system (Hokanson E20 and AG101, D.E. Hokanson Inc., Bellevue, WA, USA) to the higher of 60 mmHg above systolic BP or 200 mmHg. Following deflation, PAT signals were recorded for 5 min. The ratio of reactive hyperaemic response (taken as the average amplitude of the PAT signal 90–150 s after cuff deflation) to basal flow (taken as average PAT amplitude over 3.5 min), indexed to the contralateral control arm, was calculated. A reactive hyperaemia index $\leq$ 1.67 was considered abnormal.

Circulating biomarkers of endothelial health included asymmetric dimethylarginine (ADMA),[13,14] F2-isoprostanes,[15] growth differentiation factor-15 (GDF-15),[16] and von Willebrand factor (vWF).[17] ADMA and GDF-15 were quantitated via commercially available enzyme-linked immunosorbent assay (ELISA) kits (Immundiagnostik and R&D Systems respectively). F2-isoprostanes were measured using Enzo-Life competitive assays. Quantitative determination of vWF antigen (vWF:Ag) in plasma was undertaken using STA-Liatest immune-turbidimetric assays. Concurrently, levels of N-terminal fragment of the prohormone brain natriuretic peptide (NT-proBNP) were quantified using standard ROCHE Elecsys system assays.[18]

Comprehensive two-dimensional and Doppler echocardiographies were performed by trained research echocardiographers using state-of-the-art ultrasound machines available at all sites. Standard transthoracic echocardiographic views and tissue Doppler measurements were obtained according to the American Society of Echocardiography Guidelines.[19] Measurements of particular interest included left ventricular (LV) volume, LV mass, left atrial volume, mitral E/e' ratio, and pulmonary artery systolic pressure (estimated from tricuspid regurgitation signal and inferior vena cava characteristics). A central core lab for echocardiography and vascular ultrasound was established at the National University Heart Center to ensure standardization of data acquisition and analysis.

Renal function is expressed as eGFR, in mL/min/1.73 m^2; eGFR was derived using CKD-Epidemiology Collaboration equation using creatinine methods traceable to isotope dilution mass spectrometry.[20,21] Urine protein–creatinine ratio and plasma neutrophil gelatinase-associated lipocalin (NGAL) were used as markers of kidney injury.[22] Early-morning spot urine was tested for protein and creatinine concentrations. Assays were performed on the Siemens Advia 2400. Urine protein was measured using a pyrogallol-based assay calibrated to the manufacturer's internal standard, and creatinine was measured by an enzymatic method with the assay calibrated with manufacturer-provided materials traceable to standardized creatinine measured by isotope dilution mass. Plasma NGAL measurements were done using R&D Systems Quantikine ELISA (colorimetric) assays.

HRQOL was assessed via the 36-item Short Form Health Survey (SF-36), which is a validated self-administered questionnaire providing a multi-dimensional generic measure of HRQOL. The SF-36 contains 36 items measuring eight dimensions of health and well-being: physical functioning, role limitations due to physical problems, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health. Each dimension was scored from 0 (worst possible health state) to 100 (best possible health state) by coding, summating, and transforming its relevant item scores according to the SF-36 manual.[23]

All-cause mortality and HF hospitalization were regarded as a composite event.

Safety was assessed based on serious and non-serious adverse events that occurred during the study or within 2 weeks of final visit. Adverse events of special interest included headache, dizziness, rash, hypotension (systolic BP $<$ 100 mmHg), infection, worsening renal failure (an increase in serum creatinine by 26.5 mmol/L, or 20% drop in eGFR), arrhythmias, HF hospitalizations, and all-cause mortality. These events were reported by clinical investigators using accelerated reporting timelines and were based on self-reported symptoms by study subjects or hospital records.

Statistical analysis

Sample size was estimated based on the difference between mean change in 6MWT distance between H-ISDN and standard therapy groups. A difference of 45 m was considered clinically meaningful difference.[24] A sample size of 160 subjects (80 in each arm) will be needed for a confirmatory study (phase III) to detect a difference of 45 m in 6MWT between groups to achieve 80% power at a 5% two-sided type I error rate, assuming a standard deviation (SD) of 100 m. Originally designed as a phase III study, significant difficulties and delays with recruitment prompted a re-evaluation of the sample size. We thus planned our study with 25% of the sample size required for the confirmatory study, following the suggestions of Fleming & Richardson for proof-of-concept (phase II) studies.[25] That is, planned sample size was 44 subjects (22 in each arm), after accounting for 10% attrition rate.

Unadjusted mean change in 6MWT at 6 months between groups was compared using an ordinary linear regression model for 6MWT at 6 months adjusted for its baseline value. The analysis was then adjusted for baseline values of NT-proBNP levels, aetiology of cardiomyopathy and atrial fibrillation, and treatment compliance rate during the trial. A similar analysis was performed for other efficacy endpoints. Efficacy endpoints with highly skewed distribution were analysed using median regression model with same covariates as the ordinary lease square regression model. Safety endpoints are presented using counts and percentages using the treated population. Both efficacy and safety analyses were performed based on the intention-to-treat population, as all subjects received at least one dose of planned treatments in the study.

Results

From January 2015 to July 2018, a total of 1631 subjects were assessed for eligibility at three sites; a total of 44 subjects were randomized. The Consolidated Standards of Reporting Trials flow chart is presented in *Figure 1*. Majority of those excluded failed to meet the inclusion criteria—747 (50%) did not have renal dysfunction, 463 (31%) were not hospitalized for HF in the preceding year, 269 (18%) were already receiving nitrates or hydralazine, and 254 (17%) had end-stage renal disease, with eGFR $<$ 15 mL/min/m^2.

There was no withdrawal or loss to follow-up over the 26 week duration of study. There was one mortality from the standard-of-care group. Data from 43 subjects contributed to the outcome analysis of the trial.

The overall compliance to H-ISDN was 79%. Fifteen (68%) subjects achieved at least 80% compliance rate. Three (14%) reported compliance of $<$60%. The main reason for poor compliance was pill burden, given the frequency of dosing and number of pills.

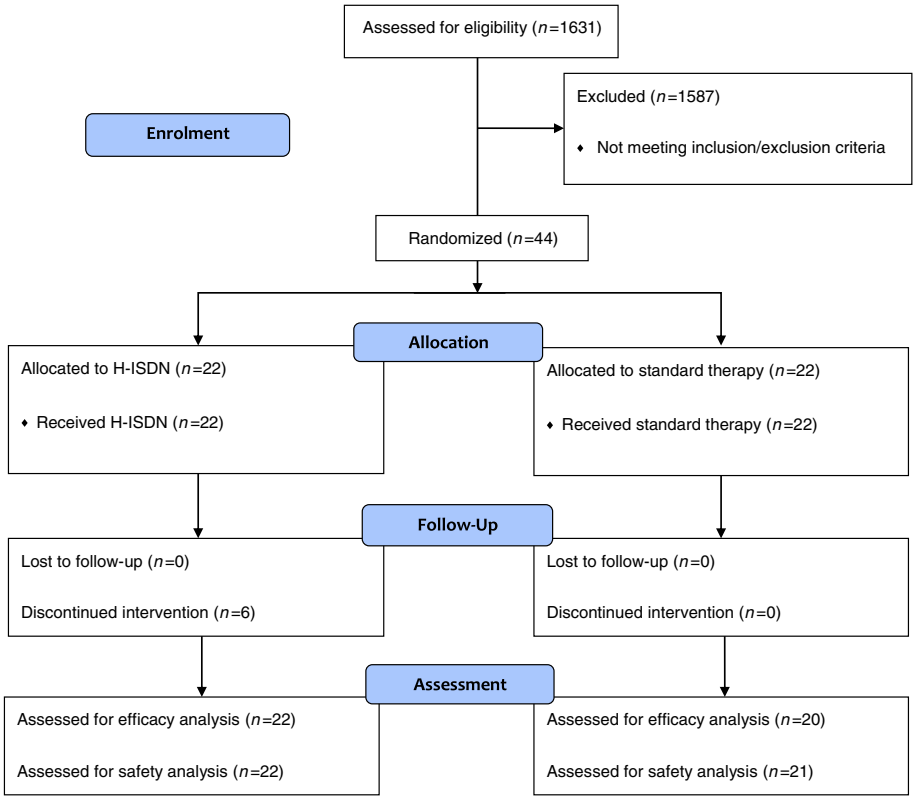
Baseline characteristics

The groups were comparable in terms of gender, ethnicity, markers of endothelial function, and medication use (*Table 1*). Although the H-ISDN group was younger, they had more co-morbidities in the form of peripheral vascular disease, chronic respiratory disease, obesity (higher body mass index), smoking, and alcohol use. There was a greater proportion of non-ischaemic cardiomyopathy amongst the H-ISDN group; they also had higher levels of NT-proBNP and of renal injury biomarkers and worse 6MWT distance.

Efficacy endpoints

The 6MWT distance increased from mean 264 (SD 107) to 291 (SD 92) with H-ISDN; it changed from 297 (SD 103) to 288 (SD 104) with standard of care. Unadjusted analysis for the change in 6MWT distance over 24 weeks showed a mean difference between groups of 27 m in favour of H-ISDN (95% CI: $-$12 to 66). Following adjustment for treatment compliance and baseline values of NT-proBNP levels, aetiology of cardiomyopathy, and atrial fibrillation, the mean difference in 6MWT distance between groups was 16 m (95% CI: $-$36 to 68). There were little differences in renal, cardiac, and endothelial functions; loop diuretic requirements; or SF-36 scores. *Table 2* summarizes the results of efficacy endpoints.

Figure 1 Patient flow chart. Abbreviation: H-ISDN, hydralazine–isosorbide dinitrate.



Abbreviation: H-ISDN, hydralazine-isosorbide dinitrate

Safety endpoints

All patients assigned to H-ISDN received at least one dose of the study treatment; they were included in the safety analysis. Headache or giddiness, and hypotension occurred more with H-ISDN compared with standard of care [5 (23%) vs. 0 (0%), and 4 (18%) vs. 1 (4%), respectively] (*Table 3*). Side effects with H-ISDN resulted in six (27%) discontinuing with the therapy, two of whom discontinued within the first week. One patient developed exanthematous drug eruption with H-ISDN, which was attributed to the hydralazine component. Her symptoms completely resolved 2 days after discontinuation of the drug.

A total of 13 patients experienced HF readmissions over the course of the study: five (23%) in the H-ISDN group and eight (36%) in the standard-of-care group. One death occurred in the standard-of-care group.

Discussion

The NICHE proof-of-concept study is the first prospective randomized controlled trial to compare the effect of fixed-dose H-ISDN in an outpatient cohort of older and sicker Southeast Asian patients with CRS. Over a period of 6 months, H-ISDN improved functional capacity, as measured using the 6MWT, compared with standard of care, but this effect was attenuated following adjustment for baseline covariates. While under-powered for definitive conclusions, we did not find meaningful differences in endothelial and renal function, cardiac structure and function, and QOL. Compliance to H-ISDN was sub-optimal, and side effects were prevalent leading to the discontinuation of H-ISDN in a proportion of subjects.

The rationale for benefit of H-ISDN combination in CRS lies in the notion of CRS as a state of abnormal neurohormonal activation with subsequent impairment of the L-arginine–NO pathway, culminating in endothelial dysfunction.[26,27] The synergistic combination of H-ISDN, through its effects on the endothelium, is thought to benefit patients with CRS, a theory of particular relevance amongst our patients, as evident by the derangements in endothelial biomarkers. By enhancing NO bioavailability, ISDN enhances inotropy and lusitropy through the NO–soluble guanylate cyclase–cyclic guanosine monophosphate signalling pathway.[28] Additional effects of ISDN include venodilation at lower concentrations and

Table 1 Characteristics of patients at baseline

Characteristic	H-ISDN (n = 22)	Standard of care (n = 22)
Age (years)—mean (SD)	70.2 (10.1)	72.3 (11.2)
Male sex—*n* (%)	16 (72.7)	17 (77.3)
Ethnic group—*n* (%)		
Chinese	11 (50.0)	13 (59.1)
Malay	9 (40.9)	9 (40.9)
Indian	2 (9.1)	0 (0.0)
Medical history—*n* (%)		
Hypertension	19 (86.4)	20 (90.9)
Diabetes	12 (54.5)	15 (68.2)
Coronary artery disease	12 (54.5)	16 (72.7)
Atrial fibrillation	7 (31.8)	10 (45.6)
Peripheral vascular disease	2 (9.1)	0 (0.0)
Chronic respiratory disease	2 (9.1)	0 (0.0)
Cerebrovascular accident	2 (9.1)	3 (13.6)
Smoker, ex or current	14 (63.6)	8 (36.4)
Alcohol history	5 (22.7)	3 (13.6)
BMI (kg/m^2)—mean (SD)	26.9 (5.9)	25.9 (5.0)
SBP (mmHg)—mean (SD)	139.8 (23.6)	147.0 (18.5)
HR (beats/min)—mean (SD)	72 (15)	71 (12)
Clinical features of HF		
Ischaemic cardiomyopathy—*n* (%)	16 (72.7)	21 (95.5)
NYHA class III/IV—*n*(%)	7 (31.8)	6 (27.3)
NT-proBNP (pg/mL)—median (IQR)	1474.5 (1990.4)	890.5 (1307.4)
Cardiac structure and function		
LVEF (%)—mean (SD)	43.2 (12.4)	40.1 (11.7)
LVEDV_Indexed (mL/m^2)—mean (SD)	72.2 (22.3)	72.0 (24.8)
LVESV_Indexed (mL/m^2)—mean (SD)	42.6 (19.4)	45.2 (23.9)
LAV_Indexed (mL/m^2)—mean (SD)	54.9 (20.3)	53.3 (19.4)
Mitral E/e′—mean (SD)	21.7 (11.8)	16.5 (4.0)
PASP (mmHg)—mean (SD)	38.4 (18.9)	31.2 (10.9)
Endothelial function		
RHI—mean (SD)	1.76 (0.46)	1.98 (0.85)
ADMA (μmol/L)—mean (SD)	0.66 (0.20)	0.65 (0.18)
F2-isoprostanes (pg/mL)—mean (SD)	3301 (841)	3066 (610)
GDF-15 (pg/mL)—mean (SD)	2964 (1439)	2886 (1561)
vWF (%)– mean (SD)	209.5 (99.9)	201.2 (68.7)
Renal function		
Estimated GFR (mL/min)—mean (SD)	50.3 (17.7)	43.6 (11.3)
NGAL (ng/mL)—mean (SD)	119.1 (51.9)	109.8 (44.6)
Urine PCR—median (IQR)	24.0 (86.0)	22.5 (37.0)
SF-36 HRQOL score—mean (SD)		
Physical functioning	58.9 (25.6)	57.3 (19.8)
Role limitation due to physical health	53.4 (37.2)	59.1 (40.5)
Role limitation due to emotional problems	75.8 (37.3)	69.7 (38.4)
Energy/fatigue	57.5 (23.9)	57.3 (15.3)
Emotional well-being	73.5 (25.3)	78.4 (17.4)
Social functioning	79.0 (23.3)	73.3 (26.5)
Pain	77.8 (26.8)	82.7 (16.0)
General health	61.8 (20.3)	64.3 (16.0)
Treatment at randomization—*n* (%)		
Loop diuretic (frusemide)	19 (86.4)	21 (95.5)
≤40 mg/day	17 (77.3)	16 (72.7)
41–80 mg/day	1 (4.6)	4 (18.2)
>80 mg/day	1 (4.6)	1 (4.6)
Beta-blocker	21 (95.5)	20 (90.9)
ACE-I/ARB	15 (68.2)	19 (86.4)
MRA	8 (36.4)	11 (50.0)
CCB	8 (36.4)	8 (36.4)
Digoxin	6 (27.3)	1 (4.6)
CRT	1 (4.6)	1 (4.6)
ICD	1 (4.6)	1 (4.6)
6MWT (m)—mean (SD)	263.9 (107.0)	296.6 (102.8)

ACE-I, angiotensin-converting enzyme inhibitor; ADMA, asymmetric dimethylarginine; ARB, angiotensin II receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CRT, cardiac resynchronization therapy; GDF-15, growth differentiation factor 15; GFR, glomerular filtration rate; HF, heart failure; H-ISDN, hydralazine–isosorbide dinitrate; HR, heart rate; ICD, implantable cardiac defibrillator; IQR, inter-quartile range; LAV_Indexed, left atrial volume indexed to body surface area; LVEDV_Indexed, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESV_Indexed, left ventricular end-systolic volume indexed to body surface area; MRA, mineralocorticoid receptor blocker; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro B type natriuretic peptide; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; PCR, protein–creatinine ratio; RHI, reactive hyperaemia index; SBP, systolic blood pressure; vWF, von Willebrand factor.

Table 2 Primary and secondary outcomes

	Unadjusted mean difference (95% CI)[a]	Unadjusted mean difference (95% CI)[b]	Adjusted mean difference (95% CI)[c]	Adjusted mean difference (95% CI)[d]
6MWT (m)	27.3 (−11.6, 66.3)	28.1 (−18.7, 74.9)	19.0 (−21.7, 59.6)	16.0 (−35.9, 68.0)
Renal function				
eGFR (mL/min)	−1.0 (−6.9, 4.9)	0.1 (−6.5, 6.7)	−0.5 (−6.8, 5.8)	0.6 (−6.9, 8.1)
NGAL (ng/mL)	0.9 (−15.8, 17.7)	−1.6 (−21.9, 18.8)	4.0 (−13.1, 21.2)	4.9 (−18.0, 27.8)
uPCR*	2.5 (−26.5, 31.4)	2.5 (−26.4, 31.3)	5.2 (−117.5, 127.9)	27.3 (−109.2, 163.8)
Cardiac structure and function				
LVEF (%)	2.2 (−4.5, 8.9)	2.6 (−5.3, 10.6)	−0.7 (−8.0, 6.7)	−1.4 (−10.9, 8.2)
LVEDV_Indexed (mL/m^2)	1.5 (−10.2, 13.1)	2.7 (−11.7, 17.1)	1.7 (−11.3, 14.6)	3.4 (−14.1, 20.8)
LVESV_Indexed (mL/m^2)	−0.7 (−9.8, 8.3)	0.2 (−10.5, 11.0)	1.3 (−8.8, 11.5)	3.7 (−9.3, 16.7)
LAV_Indexed (mL/m^2)	11.4 (−0.8, 23.7)	11.4 (−3.6, 26.4)	7.9 (−5.0, 20.9)	6.0 (−10.6, 22.5)
Mitral E/e′	−3.0 (−9.1, 3.2)	−0.4 (−7.4, 6.5)	−2.0 (−8.7, 4.7)	2.8 (−4.1, 9.6)
PASP (mmHg)	−1.8 (−10.8, 7.2)	0.6 (−11.5, 12.6)	0.7 (−10.1, 11.4)	7.8 (−9.6, 25.2)
NT-proBNP (pg/mL)*	228.2 (−629.9, 1086.4)	132.5 (−775.4, 1040.4)	563.9 (−241.9, 1369.6)	554.3 (−365.5, 1474.1)
Endothelial biomarkers				
RHI				
ADMA (μmol/L)	−0.002 (−0.135, 0.130)	−0.037 (−0.202, 0.127)	0.0257 (−0.113, 0.165)	−0.0002 (−0.184, 0.183)
vWF (%)	4.75 (−19.1, 28.6)	6.4 (−23.4, 36.2)	4.9 (−19.9, 29.7)	5.9 (−27.3, 39.1)
F2-isoprostanes (pg/mL)	148.2 (−303.6, 600.1)	269.6 (−228.2, 767.3)	257.4 (−249.7, 764.5)	478.5 (−105.9, 1062.9)
GDF-15 (pg/mL)	383.3 (−348.5, 1115.0)	233.0 (−658.5, 1124.6)	631.2 (−153.6, 1416.1)	533.2 (−522.2, 1588.5)
SF-36 HRQOL scores				
General health	−2.9 (−13.1, 7.4)	−5.1 (−15.0, 4.7)	−4.7 (−15.8, 6.4)	−5.3 (−16.3, 5.8)

6MWT, 6 min walk test; ADMA, asymmetric dimethylarginine; CI, confidence interval; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HF, heart failure; H-ISDN, hydralazine–isosorbide dinitrate; LAV_Indexed, left atrial volume indexed to body surface area; LVEDV_Indexed, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESV_Indexed, left ventricular end-systolic volume indexed to body surface area; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; RHI, reactive hyperaemia index; SF-36, Short Form-36; uPCR, urine protein–creatinine ratio; vWF, von Willebrand factor.
[a]Mean difference for change in outcome at 6 months from baseline between H-ISDN and standard of care.
[b]Mean difference for change in outcome at 6 months from baseline between H-ISDN and standard of care, adjusted for compliance.
[c]Mean difference for change in outcome at 6 months from baseline between H-ISDN and standard of care, adjusted for baseline, NT-proBNP, aetiology of HF, and atrial fibrillation.
[d]Mean difference for change in outcome at 6 months from baseline between H-ISDN and standard of care, adjusted for baseline, NT-proBNP, aetiology of HF, atrial fibrillation, and compliance.
*Reported medians instead of means based on the median regression model due to skewed nature of the data.

Table 3 Adverse events

Event, *n* (%)	H-ISDN (*n* = 22)	Standard of care (*n* = 22)
Symptoms		
Headache/giddiness	5 (22.7)	0 (0)
Rash	1 (4.5)	1 (4.5)
Hypotension	4 (18.2)	1 (4.5)
Infection	3 (13.6)	3 (13.6)
WRF	2 (9.1)	1 (4.5)
Arrhythmias	3 (13.6)	1 (4.5)
HF hospitalization	5 (22.7)	8 (36.4)
Mortality	0	1 (4.5)

HF, heart failure; H-ISDN, hydralazine–isosorbide dinitrate; WRF, worsening renal function.
n (%) refer to number and proportion of subjects, respectively.

arterial vasodilatation at higher concentrations—the former reduces preload and alleviates pulmonary congestion, while the latter improves pulmonary pressures, LV afterload, and subendocardial perfusion. On its own, hydralazine is an arterial vasodilator and has powerful peroxynitrite-quenching properties.[29] When added to ISDN, hydralazine prevents the development of nitrate tolerance.[30,31] In keeping with these mechanisms, Massie and colleagues showed H-ISDN reduced LV filling pressures, increased cardiac index, and reduced systemic vascular resistance in HFrEF patients.[32]

Our findings are contrary to the theoretical benefits of H-ISDN and prior studies in HFrEF, with neutral findings consistent with real-world data and studies in HFpEF.[8–10,33] In particular, our findings are in agreement with real-world data on a large cohort of HFpEF patients enrolled in the Swedish Heart Failure Registry.[8] The study included a large cohort of HF with mid-range ejection fraction (HFmrEF) (defined as LV ejection fraction 40–49%) patients, who bore some similarities to our study population. Neither the HFmrEF patients nor those with CKD (eGFR 30–59 mL/min/1.73 m^2 and eGFR $<$ 30 mL/min/1.73 m^2) benefitted from nitrate administration in the sub-group analyses.

There are several possible explanations for our neutral findings. Firstly, our pilot study was under-powered. Secondly, our patients were elderly and rather ill, with 90% on chronic loop diuretic use, a median NT-proBNP level of 1345 pg/mL, and a high prevalence of co-morbidity. It is conceivable that the severity and progression of disease in our patients may have been beyond the modifiable stage. Indeed,

the lack of improvements in endothelial, cardiac, and renal functions despite therapy is in keeping with irreversibility. Thirdly, the majority of our patients were on beta-blockers and renin–angiotensin system (RAS) antagonists. Both classes of drugs have been shown to improve endothelial function,[34,35] and the addition of H-ISDN on top of these may not further improve endothelial function, even if the disease was modifiable. Finally, the development of pseudotolerance with chronic nitrate therapy, with reflex neurohormonal activation, not only reversed the initial haemodynamic benefits but may have been poorly tolerated in our elderly study population.

Our elderly study population with co-morbidities and polypharmacy were susceptible to drug interactions and adverse reactions. Their attenuated baroreceptor reflexes would render them sensitive to volume changes and hypotension. Despite using lower doses of H-ISDN compared with those in the V-HEFT trials, adverse effects were prevalent in the H-ISDN group; this, coupled with poor compliance, supports the notion that H-ISDN may not have been well tolerated. This is consistent with the NEAT-HFpEF trial and real-world settings, where it was postulated that adverse side effects of high-dose nitrates may have limited potential clinical benefits in patients.

Few evidence-based therapies offer both cardiovascular and renal protection. Combination therapy with H-ISDN, despite its theoretical benefits, improved neither cardiac structure and function nor renal function in our study. There are, however, promising therapies for CRS. The combination of sacubitril and valsartan (LCZ696), which was shown to be superior to enalapril in reducing HF hospitalization and cardiovascular mortality in HFrEF patients,[36] was found to attenuate the decline in renal function in chronic HF patients, regardless of baseline renal function.[37] More recently, sodium-glucose cotransporter-2 (SGLT-2) inhibitors have been shown to prevent HF,[38–40] improve cardiovascular outcomes in proven HFrEF,[41] and delay the progression of CKD in patients with and without HF.[42] Cardiovascular benefits and renal protection appear to be interlinked, and several ongoing trials investigating cardiorenal outcomes with SGLT-2 inhibitors may provide further mechanistic insights.

This study was originally designed as a phase III trial. Significant difficulties and delays with recruitment prompted a re-evaluation and eventual reduction of the sample size. The poor recruitment may be partly explained by the inclusion and exclusion criteria that were rather specific, resulting in a small pool of potential subjects for enrolment. Furthermore, there may be sociocultural issues related to trial participation in the local context. This is particularly relevant in our study, as the potential subjects were older; it is conceivable that the scientific literacy is low in this group. Lastly, there were other ongoing HF trials, resulting in competition for the same pool of patients.

The strengths of our study included comprehensive phenotyping of endothelial, renal, and cardiac function in our study population. No subject was loss to follow-up, and all data were used for analyses. Our study was limited by its single-blind, open-label design where only the outcome assessor was blinded to the allocation. Although the primary outcome was an objective measure, placebo effect could not be excluded. There were imbalances between study groups despite best efforts to standardize randomization—H-ISDN group had shorter 6MWT distances at baseline, higher mean NT-proBNP levels, worse endothelial function, and lesser use of RAS antagonists. Nevertheless, sensitivity analysis was performed adjusting baseline characteristics and found results consistent with unadjusted analysis.

Conclusions

Results from this pilot study, in which recruitment and compliance rates were lower than expected, do not support the addition of H-ISDN on the top of standard medical therapy to improve exercise capacity in patients with CRS. Furthermore, the addition of H-ISDN did not improve cardiac, renal, and endothelial functions or HRQOL in CRS.

Conflict of interest

No conflict of interest is directly relevant to present work. SLL is supported by the National University Health System Clinician Scientist Program; she has received research grants from National University Health System, National Kidney Foundation of Singapore, and Singapore Heart Foundation. HRC is supported by a Transitional Award from the National Medical Research Council Singapore; he has received research grants from National University Health System, National Kidney Foundation of Singapore, National Medical Research Council, and Baxter. AMR is supported by a Senior Translational Research (STaR) award from the National Medical Research Council of Singapore; holds the New Zealand Heart Foundation Chair of Cardiovascular Studies; has received research support from Boston Scientific, Bayer, AstraZeneca, Medtronic, Roche Diagnostics, Abbott Laboratories, Thermo Fisher, and Critical Diagnostics; and has consulted for Bayer, Novartis, Merck, AstraZeneca, and Roche Diagnostics. CSPL is supported by a Clinician Scientist Award from the National Medical Research Council Singapore. She has received research support from Boston Scientific, Medtronic, and Vifor Pharma and has consulted for Bayer, Novartis, Takeda, Merck, AstraZeneca, Janssen Research & Development, and Menarini. She has served on the clinical endpoint committee for DC Devices.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Trial inclusion and exclusion criteria.

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