Isosorbide Dinitrate, With or Without Hydralazine, Does Not Reduce Wave Reflections, Left Ventricular Hypertrophy, or Myocardial Fibrosis in Patients With Heart Failure With Preserved Ejection Fraction

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Background—Wave reflections, which are increased in patients with heart failure with preserved ejection fraction, impair diastolic function and promote pathologic myocardial remodeling. Organic nitrates reduce wave reflections acutely, but whether this is sustained chronically or affected by hydralazine coadministration is unknown.

Methods and Results—We randomized 44 patients with heart failure with preserved ejection fraction in a double-blinded fashion to isosorbide dinitrate (ISDN; n=13), ISDN+hydralazine (ISDN+hydral; n=15), or placebo (n=16) for 6 months. The primary end point was the change in reflection magnitude (RM; assessed with arterial tonometry and Doppler echocardiography). Secondary end points included change in left ventricular mass and fibrosis, measured with cardiac magnetic resonance imaging, and the 6-minute walk distance. ISDN reduced aortic characteristic impedance (mean baseline=0.15 [95% CI, 0.14–0.17], 3 months=0.11 [95% CI, 0.10–0.13], 6 months=0.10 [95% CI, 0.08–0.12] mm Hg/mL per second; P=0.003) and forward wave amplitude (Pf, mean baseline=54.8 [95% CI, 47.6–62.0], 3 months=42.2 [95% CI, 33.2–51.3]; 6 months=37.0 [95% CI, 27.2–46.8] mm Hg, P=0.04), but had no effect on RM (P=0.64), left ventricular mass (P=0.33), or fibrosis (P=0.63). ISDN+hydral increased RM (mean baseline=0.39 [95% CI, 0.35–0.43]; 3 months=0.31 [95% CI, 0.25–0.36]; 6 months=0.44 [95% CI, 0.37–0.51], P=0.03), reduced 6-minute walk distance (mean baseline=343.3 [95% CI, 319.2–367.4]; 6 months=277.0 [95% CI, 242.7–311.4] meters, P=0.022), and increased native myocardial T1 (mean baseline=1016.2 [95% CI, 1002.7–1054.5], 6 months=1054.5 [95% CI, 1036.5–1072.3], P=0.021). A high proportion of patients experienced adverse events with active therapy (ISDN=61.5%, ISDN+hydral=60.0%; placebo=12.5%; P=0.007).

Conclusions—ISDN, with or without hydralazine, does not exert beneficial effects on RM, left ventricular remodeling, or submaximal exercise and is poorly tolerated. ISDN+hydral appears to have deleterious effects on RM, myocardial remodeling, and submaximal exercise. Our findings do not support the routine use of these vasodilators in patients with heart failure with preserved ejection fraction.

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Key Words: heart failure • hemodynamics • magnetic resonance imaging • remodeling heart failure • vascular biology • vascular stiffness • vasodilators

Heart failure with preserved ejection fraction (HFpEF) is an epidemic condition for which the underlying mechanisms are incompletely understood. Epidemiologic data demonstrate a greater prevalence of hypertension, advanced age, and vascular risk factors such as renal dysfunction and diabetes, in patients with HFpEF,1–4 all of which increase...
vascular stiffness and pulsatile loading on the left ventricle. Observational data demonstrate increased pulsatile load and wave reflections in patients with HFrEF, which correlate with decreased exercise capacity, a fundamental feature of the disease.

Wave reflections, arising from the gradual increase in impedance along the arterial tree and from discrete sites of impedance mismatch, augment late systolic load on the left ventricle. Late systolic load increases left ventricular (LV) mass and fibrosis and worsens diastolic function in animal models. Late systolic load is also associated with increased LV mass and geometry and impaired systolic and diastolic function in humans. Furthermore, the reduction in reflection magnitude (RM) correlates with reductions in LV mass during antihypertensive therapy.

Organic nitrates have been shown to blunt wave reflections in short-term studies, although tolerance remains a concern with chronic administration. However, therapy with hydralazine has been shown to attenuate this tolerance. Whether organic nitrates can modulate wave reflections chronically and whether this effect is modulated by hydralazine is unknown. Furthermore, the effects of long-term nitrate therapy on arterial hemodynamics, LV remodeling, diastolic function, and exercise capacity in patients with HFrEF are unknown. We designed the current pilot trial to test whether the organic nitrate isosorbide dinitrate (ISDN), with or without hydralazine, blunts wave reflections, thereby leading to improvements in myocardial structure and function.

Methods

Inclusion/Exclusion Criteria

Inclusion criteria included symptomatic heart failure with a preserved ejection fraction (LV ejection fraction >50%), in addition to at least one of the following: (1) prior hospitalization for decompensated heart failure; (2) acute treatment for heart failure requiring intravenous diuretics or hemofiltration; (3) echocardiographic evidence for elevated filling pressures; (4) chronic treatment with a loop diuretic for control of symptoms; (5) or an elevated N-terminal pro-brain natriuretic peptide (NT-pro-BNP) level. Patients were required to be on stable medical therapy for the past month. Exclusion criteria included any rhythm other than sinus with native conduction; noncardiac conditions that significantly limited exercise (orthopedic or neuromuscular); known hypertrophic, infiltrative, or inflammatory cardiomyopathy; pericardial disease; significant pulmonary disease; primary pulmonary arteriopathy; acute coronary syndrome or coronary revascularization within the past 60 days; clinically significant perfusion defects on stress imaging without subsequent revascularization; significant valvular disease (eg, moderate or greater mitral regurgitation or aortic stenosis); uncontrolled hypertension (systolic blood pressure >180 mm Hg or diastolic blood pressure >100 mm Hg); prior reduced LV ejection fraction <50%; hemoglobin <10 g/dL; current therapy with organic nitrates or hydralazine; and elevations on liver function test results. Additional exclusion criteria for the cardiac magnetic resonance imaging (MRI) included impaired renal function precluding the administration of gadolinium (estimated glomerular filtration rate <30 mL/min per 1.73 m²) and significant claustrophobia.

Study Design

This was a randomized double-blinded pilot clinical trial of ISDN (40 mg 3 times daily), ISDN plus hydralazine (ISDN 40 mg+hydral 75 mg, 3 times daily), or placebo (PB). The doses selected were based on A-HeFT (the African-American Heart Failure Trial), which demonstrated a benefit with these doses of ISDN+hydral in black patients with HFrEF. We tested the hypothesis that chronic administration of ISDN, with or without hydralazine, would blunt wave reflections. Our primary end point was the change in RM, assessed 6 months after study initiation. Key secondary end points included change in LV mass and diffuse interstitial myocardial fibrosis measured by MRI, change in 6-minute walk (6MW) distance, change in diastolic function, change in NT-pro-BNP, and change in quality of life assessed by the Kansas City Cardiomyopathy Questionnaire. This protocol was approved by the institutional review boards of the Philadelphia Veterans Affairs Hospital and the Hospital of the University of Pennsylvania. All patients provided written informed consent. The trial was registered at ClinicalTrials.gov (www.ClinicalTrials.gov, NCT01516346).

After randomization, patients were started on half of the intended target dose. After 1 week, patients were reassessed, and the doses of study medications were increased unless orthostatic hypotension or other limiting side effect was present. Study medications were continued for 6 months. The first patient was enrolled in March 2012, and the final study visit was conducted in December 2015.

Cardiac MRI—LV Structure and Function

Participants underwent a cardiac MRI examination at baseline and at 6 months to assess LV structure and function using a 1.5 Tesla (T) whole-body MRI scanner (Avanto or Espree, Siemens Healthcare, Malvern, Pennsylvania) equipped with a phased-array cardiac coil. LV volumes and ejection fractions were determined using balanced steady-state free-precession cine imaging. Typical parameters were as follows: repetition time=2.6 ms; echo time=1.3 ms; phases=30; slice thickness=8 mm; bandwidth=898 Hz/pixel, flip angle=70°, field of...
view=300 to 340 mm², matrix size=192×192; and parallel imaging factor=2. LV short-axis stack cine images were manually traced at end-diastole and end-systole using CMR42 software (Circle CVI, Calgary, Alberta, Canada). LV mass was computed as the difference between epicardial and endocardial volumes, multiplied by myocardial density, and was measured at end-diastole and end-systole with the results averaged. LV mass was normalized for height in meters raised to the power of 1.7.29 Stroke volume was computed as the difference between the end-diastolic and end-systolic volumes. End-diastolic volume (EDV) and stroke volume were indexed to body surface area.

We used a modified Look-Locker inversion recovery (MOLLI) sequence to assess T1 times prior to and following the intravenous administration of gadolinium contrast (gadopentetate dimeglumine, 0.15 mmol/kg or equivalent) in a midventricular short-axis slice.30,31 Scan parameters for MOLLI were: field of view=340 mm²; matrix size=144×192; slice thickness=6 mm; repetition time=2.4 ms; echo time=1.18 ms; flip angle=30°; bandwidth=1000 Hz/pixel; and parallel imaging=2. Myocardial T1 measurements were performed before and at several time points (≥5, 10, 15, and 20–40 minutes) after gadolinium administration. MOLLI was performed with a 5-3-3 schema (2 inversions, 5 TIs after inversion 1, 3 T1 recovery heartbeats, and 3 TIs after inversion 2). All available blood and myocardial T1 measurements were used to compute lambda (λ, the myocardium-blood partition coefficient) as the slope of the myocardial
1/T1 over the blood 1/T1 change, via linear regression. The percent of myocardial tissue comprised by the extracellular space (extracellular volume fraction [ECV], %) = k \times (1/\text{hematocrit}). As heart rate correction did not appreciably affect the results, only the noncorrected values are presented.

Echocardiography and Arterial Tonometry

Echocardiography with arterial tonometry was performed at baseline, after 3 months, and at the final 6-month visit. Echocardiography was performed using a Vivid e9 or Vivid I machine (General Electric, Fairfield, CT). Diastolic function was assessed according to American Society of Echocardiography criteria. Each metric was quantified in triplicate with average values presented. Left atrial volume was quantified using the area-length method and indexed to body surface area (left atrial volume index). Volumetric flow was quantified using pulse-wave Doppler measurements from the left ventricular outflow tract in the 5-chamber view and the left ventricular outflow tract cross-sectional area computed from its diameter measured in the parasternal long-axis view.

Applanation tonometry was performed at the carotid, radial, and femoral arteries using a high-fidelity tonometer (Millar Instruments, Houston, TX), with a single-lead ECG used as a fiducial point. Surface measurements were obtained from the sternal notch to the site of interrogation at the carotid and femoral arteries to compute pulse wave velocity. Radial tonometry was calibrated using the brachial systolic blood pressure and diastolic blood pressure, obtained using a validated oscillometric device (Omron HEM-705CP, Omron Corp, Kyoto, Japan or Accutorr Plus, Datascype Corp., Paramus, NJ). Mean arterial pressure was computed as the mean pressure from the radial pressure waveform. Carotid tonometry, calibrated using mean arterial pressure and
Table 1. Baseline Demographic, Medication, Laboratory, and Imaging Data

| Variable                                      | All Participants (N=44) | ISDN (n=13)  | ISDN+Hydral (n=15) | Placebo (n=16) |
|-----------------------------------------------|-------------------------|--------------|-------------------|----------------|
| Age, median (IQR), y                         | 62 (59–68)              | 61 (56–65)   | 60 (55–66)        | 66.5 (59.5–72) |
| Male, No. (%)                                 | 31 (70.5)               | 8 (61.5)     | 11 (73.3)         | 12 (75)        |
| Race, No. (%)                                 |                         |              |                   |                |
| Black                                         | 27 (61.4)               | 8 (61.5)     | 10 (66.7)         | 9 (56.3)       |
| White                                         | 16 (36.4)               | 4 (30.8)     | 5 (33.3)          | 7 (43.8)       |
| Other                                         | 1 (2.3)                 | 1 (7.7)      | 0 (0)             | 0 (0)          |
| Body mass index (kg/m²), mean (SD)            | 36.7 (6.2)              | 35.7 (6.7)   | 38.2 (5.1)        | 36.2 (6.8)     |
| Obese, No. (%)                                | 36 (81.8)               | 10 (76.9)    | 13 (86.7)         | 13 (81.3)      |
| Hypertension, No. (%)                         | 40 (90.9)               | 13 (100)     | 14 (93.3)         | 13 (81.3)      |
| Hyperlipidemia, No. (%)                       | 36 (81.8)               | 10 (76.9)    | 12 (80.0)         | 14 (87.5)      |
| Coronary artery disease, No. (%)              | 16 (36.4)               | 4 (30.8)     | 6 (40.0)          | 6 (37.5)       |
| History of atrial fibrillation/flutter, No. (%)| 5 (11.4)                | 0 (0)        | 3 (20.0)          | 2 (12.5)       |
| Diabetes, No. (%)                             | 27 (61.4)               | 11 (84.6)    | 6 (40.0)          | 10 (62.5)      |
| Obstructive sleep apnea, No. (%)              | 24 (54.6)               | 6 (46.2)     | 10 (66.7)         | 8 (50.0)       |
| Medical therapy                               |                         |              |                   |                |
| β-Blockers, No. (%)                           | 26 (59.1)               | 6 (46.2)     | 9 (60.0)          | 11 (68.8)      |
| Aspirin, No. (%)                              | 30 (68.2)               | 10 (76.9)    | 9 (60.0)          | 11 (68.8)      |
| ACEI/ARB, No. (%)                             | 29 (65.9)               | 7 (53.9)     | 10 (66.7)         | 12 (75.0)      |
| Loop diuretics, No. (%)                       | 24 (54.6)               | 6 (46.2)     | 8 (53.3)          | 10 (62.5)      |
| Mineralocorticoid receptor antagonists, No. (%)| 2 (4.6)                | 1 (7.7)      | 1 (6.7)           | 0 (0)          |
| Calcium channel blockers, No. (%)             | 19 (43.2)               | 5 (38.5)     | 6 (40.0)          | 8 (50.0)       |
| Thiazide diuretics, No. (%)                   | 16 (36.4)               | 7 (53.9)     | 6 (40.0)          | 3 (18.8)       |
| Statins, No. (%)                              | 29 (65.9)               | 10 (76.9)    | 8 (53.3)          | 11 (68.8)      |
| Baseline laboratories                         |                         |              |                   |                |
| Hematocrit, mean (SD), %                      | 38.5 (4.9)              | 39.3 (3.7)   | 38.6 (4.0)        | 37.8 (6.4)     |
| eGFR (mL/min per 1.73 m²), mean (SD)          | 70.3 (26.7)             | 77.1 (27.1)  | 70.8 (32.1)       | 64.4 (21.0)    |
| eGFR <60 mL/min per 1.73 m², No. (%)          | 18 (40.9)               | 5 (38.5)     | 7 (46.7)          | 6 (37.5)       |
| NT-pro-BNP, median (IQR), pg/mL               | 233.0 (90.5–527.0)      | 154 (88–280) | 210 (118–1190)    | 326 (83.3–720.3)|
| Elevated NT-pro-BNP, No. (%)                  | 31 (70.5)               | 9 (69.2)     | 11 (73.3)         | 11 (68.8)      |
| Baseline cMRI data                            |                         |              |                   |                |
| LV mass, median (IQR), g                      | 170.0 (132.9–200.5)     | 183.3 (136.3–215.5) | 168.8 (131.9–198.5) | 159.5 (126.6–215.0) |
| Indexed LV mass, median (IQR), g/m¹³         | 64.2 (56.6, 81.2)       | 68.0 (58.3, 87.2) | 60.2 (57.2, 73.9) | 63.6 (53.1, 80.7) |
| LVEDV, mean (SD), mL                          | 171.9 (43.8)            | 159.1 (39.3) | 176.8 (38.9)      | 179.5 (51.6)   |
| Indexed LVEDV, mean (SD), mL/m²               | 74.9 (15.2)             | 71.2 (12.8)  | 74.0 (13.6)       | 79.2 (18.4)    |
| LV ejection fraction, median (IQR), %         | 59.4 (55.1–65.5)        | 64.1 (57.8–65.9) | 56.9 (52.9–67.4) | 59.3 (55.5–65.0) |
| LVECV fraction, mean (SD), %                  | 28.9 (6.6)              | 28.3 (4.5)   | 28.6 (8.0)        | 29.6 (6.8)     |
| Native myocardial T₁, mean (SD), s            | 1013.2 (52.0)           | 1017.1 (41.3) | 1002.0 (43.2)     | 1019.9 (64.5)  |
| Baseline echocardiographic data               |                         |              |                   |                |
| Mitral E velocity, mean (SD), cm/s            | 81.0 (23.8)             | 69.4 (21.0)  | 82.2 (26.7)       | 89.8 (20.1)    |
| Mitral A velocity, mean (SD), cm/s            | 77.1 (22.6)             | 76.7 (19.7)  | 76.9 (25.2)       | 77.7 (23.8)    |

Continued
diastolic blood pressure, was used to estimate central pressures. Tonometric signals were processed using Sphygmocor software (AtCor Medical, Australia).

Central Arterial Hemodynamics

Custom-designed software was programmed using MATLAB (R2014b, MathWorks, Natick, MA) to derive input impedance, as previously described (Figure 1).33 In brief, central pressure measurements were ensemble-averaged and time-aligned with left ventricular outflow tract flow such that the upstroke of pressure and flow occurred simultaneously, peak flow was coincident with the first systolic peak or inflection point in the pressure waveform, and flow ceased at the dicrotic notch. Characteristic impedance \(Z_c\) was quantified in the frequency domain as the average modulus at higher frequencies. Total vascular resistance was quantified as the ratio of mean pressure to mean flow. Total arterial compliance was determined using the pulse pressure method.33 Linear wave separation was performed to obtain the amplitude of the forward \(P_f\) and backward \(P_b\) pressure waves. RM was defined as the ratio of \(P_b\) to \(P_f\) (\(P_b/P_f\)).

Additional Measurements Performed at Baseline and at 6 Months

The Kansas City Cardiomyopathy Questionnaire was administered at the baseline and 6-month visits.34 Basic laboratory tests, including NT-pro-BNP levels (upper limit of normal=125 pg/mL), were also performed. Patients performed a 6MW test using the standard protocol.35 The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate glomerular filtration rate.36

Statistical Methods

Baseline characteristics are presented as mean (percentage) or median (interquartile range [IQR]), as appropriate. Mixed-effects models were generated using the xtreg command in STATA (Stata/SE version 13.1, StataCorp, College Station, TX), which incorporates the correlation between repeated measurements in the same individual. No assumption of linearity was made, and all available observations were used to estimate treatment effects. An overall \(P<0.05\) was taken to be significant for each model, with post hoc comparisons between visits subsequently performed. Study end points are presented in the tables as marginal means estimated from the mixed models with 95% CIs. Due to the small number of patients who completed this pilot study, only intragroup comparisons were performed. Paired \(t\) tests using only data from the baseline and final visits were also performed for select end points. Given the small sample sizes, the signed rank test was additionally performed to demonstrate general agreement. Our study had 80% power to detect a within-group minimal change of at least 14.4 g/m^1.7 in LV mass and 0.10 in RM. Formal between-group comparisons were not performed because of the risk of type II error.

Results

The flow of patients through the study is shown in Figure 2. A total of 53 patients consented to the study. Of these 53 patients, 9 withdrew before receiving study medications; thus, 44 (83%) patients were randomized and started the study medications: 13 patients were randomized to ISDN, 15 to ISDN+hydral, and 16 to PB. Of these individuals, 30 (68%) provided 3-month central arterial hemodynamics data (ISDN=9, ISDN+hydral=9, PB=12) and 27 (61%) provided 6-month data (ISDN=7, ISDN+hydral=9, PB=11). Demographic, echocardiographic, and cardiac MRI data are shown in Table 1. The median age of patients was 62 (IQR 59–68) years. The majority of patients were obese (81.8%), hypertensive (90.9%), and had a history of diabetes (61.4%). A total of 31 (70.5%) patients had an elevated NT-pro-BNP level, and 40.9% had an estimated glomerular filtration rate <60 mL/min per 1.73 m^2. Median E/septal e’ ratio was 12.8 (IQR 10.2–15.2), and the median left atrial volume index was 29.9 (IQR 25.6–38.3) mL/m^2. Aside from the study intervention, there were no
### Table 2. Central Arterial Hemodynamics

| Marginal Means (95% CI) | ISDN | ISDN+Hydral | Placebo | Placebo |
|-------------------------|------|-------------|---------|---------|
| Baseline | 3 Months | Final | P Value | Baseline | 3 Months | Final | P Value | Baseline | 3 Months | Final | P Value |
| **Brachial** | | | | | | | | | | | |
| SBP, mm Hg | 146.0 (134.8–157.3) | 129.2 (115.1–143.2) | 123.9 (108.7–139.1) | 0.09 | 126.8 (117.9–135.7) | 126.0 (115.0–137.1) | 118.2 (105.5–130.9) | 0.56 | 138.0 (130.5–145.5) | 137.5 (128.5–146.4) | 143.0 (134.0–151.9) | 0.64 |
| **Central** | | | | | | | | | | | |
| SBP, mm Hg | 141.3 (126.9–155.7) | 113.7 (95.7–131.7) | 110.9 (91.4–130.4) | 0.051 | 122.6 (110.4–134.8) | 122.1 (105.4–138.8) | 115.3 (95.1–135.5) | 0.83 | 128.4 (121.1–135.8) | 130.1 (121.8–138.3) | 133.5 (124.8–142.3) | 0.70 |
| DBP, mm Hg | 79.2 (71.4–87.1) | 70.7 (60.9–80.5) | 72.1 (61.5–82.8) | 0.41 | 73.2 (68.3–78.2) | 72.4 (65.6–79.2) | 72.9 (64.8–81.1) | 0.98 | 69.3 (64.4–74.2) | 74.2 (68.6–79.7) | 77.1 (71.2–82.9) | 0.18 |
| MAP, mm Hg | 102.6 (93.4–111.7) | 88.4 (77.0–99.9) | 87.4 (75.0–99.8) | 0.13 | 92.7 (85.9–99.6) | 92.0 (82.5–101.4) | 91.6 (80.2–103.0) | 0.99 | 93.3 (88.2–98.5) | 95.2 (89.4–101.0) | 98.7 (92.5–104.8) | 0.47 |
| TVR, mm Hg/mL per second | 1.30 (1.09–1.52) | 1.06 (0.79–1.34) | 1.12 (0.83–1.42) | 0.42 | 1.18 (0.97–1.38) | 0.98 (0.70–1.27) | 0.94 (0.60–1.28) | 0.48 | 1.06 (0.95–1.18) | 1.09 (0.96–1.21) | 1.09 (0.96–1.22) | 0.96 |
| TAC, mL/mm Hg | 1.01 (0.84–1.18) | 1.51 (1.30–1.73) | 1.38 (1.15–1.61) | 0.01 | 1.52 (1.22–1.82) | 1.33 (0.92–1.74) | 1.50 (1.01–1.99) | 0.76 | 0.99 (0.85–1.13) | 1.16 (1.00–1.32) | 1.08 (0.92–1.25) | 0.34 |
| Z, mm Hg/mL per s | 0.15 (0.14–0.17) | 0.11 (0.10–0.13) | 0.10 (0.08–0.12) | 0.003 | 0.16 (0.09–0.22) | 0.09 (0.00–0.18) | 0.06 (−0.05 to 0.17) | 0.34 | 0.16 (0.13–0.18) | 0.13 (0.10–0.15) | 0.12 (0.09–0.15) | 0.18 |
| RM | 0.39 (0.35–0.42) | 0.41 (0.37–0.46) | 0.38 (0.33–0.43) | 0.64 | 0.39 (0.35–0.43) | 0.31 (0.25–0.36) | 0.44 (0.37–0.51) | 0.03 | 0.36 (0.30–0.41) | 0.34 (0.28–0.39) | 0.37 (0.31–0.43) | 0.75 |
| P, mm Hg | 54.8 (47.6–62.0) | 42.2 (33.2–51.3) | 37.0 (27.2–46.8) | 0.04 | 47.9 (38.6–57.2) | 42.4 (29.6–55.1) | 36.7 (21.3–52.0) | 0.53 | 55.0 (48.0–62.0) | 49.6 (41.7–57.5) | 50.8 (42.4–59.1) | 0.61 |
| P, mm Hg | 21.1 (17.4–24.7) | 17.7 (13.1–22.3) | 13.0 (8.1–18.0) | 0.08 | 18.9 (13.5–24.4) | 12.3 (4.8–19.7) | 16.0 (7.1–25.0) | 0.44 | 19.2 (16.3–22.1) | 16.5 (13.2–19.8) | 17.2 (13.8–20.7) | 0.50 |

DBP indicates diastolic blood pressure; Hydral, hydralazine; ISDN, isosorbide dinitrate; MAP, mean arterial pressure; P, magnitude of the backward wave; P, magnitude of the forward wave; RM, reflection magnitude; SBP, systolic blood pressure; TAC, total arterial compliance; TVR, total vascular resistance; Z, characteristic impedance of the ascending aorta.

*P<0.05 between baseline and the 3-month visit.
†P<0.05 between baseline and the final visits.
‡P<0.05 between the 3-month and final visits.
significant differences in medication usage between the groups (P>0.10 for all). Compliance was assessed via pill counts. In the 27 patients who completed the study, compliance rates were: ISDN: 91.8%; ISDN+hydral: 90.2%; and PB: 94.8%. The mean daily dose of ISDN in the ISDN only group was 111.4±22.7 mg. The mean ISDN and hydralazine doses in the combination group were 100.0±30.0 mg of ISDN and 187.5±56.3 mg of hydralazine. There were no differences in demographic characteristics between those who did versus those who did not complete the study (data not shown).

**Arterial Hemodynamics**

Treatment with ISDN did not reduce brachial systolic blood pressure (P=0.09), yet tended to reduce central systolic blood pressure (visit 2 versus visit 1: −27.6 [95% CI −54.0 to −1.3]; visit 3 versus visit 1: −30.4 [95% CI −58.2 to −2.6] mm Hg; overall P=0.051; Table 2), although this reduction did not reach statistical significance. There were no significant changes in brachial or central blood pressures with ISDN+hydral or PB. Heart rate and augmentation index were not significantly altered by any study medication (data not shown).

ISDN did not reduce RM, the primary end point of the study (P=0.64; Table 2). In contrast, ISDN reduced aortic Zc (P=0.003), reduced Pf (P=0.04), and increased total arterial compliance (P=0.01). Combination therapy with ISDN+hydral increased RM between the 3- and 6-month visits (P=0.012). No changes in arterial hemodynamic parameters were demonstrated in the PB group (Figure 3).

**Figure 3.** Changes in end points as compared with baseline (marginal mean differences with standard error presented). A, Reflection magnitude (RM). B, Characteristic impedance (Zc). C, Forward wave magnitude (Pf). D, Total arterial compliance (TAC). E, Six-minute walk (6MW) distance. F, Native T1 myocardial relaxation time. Hydral indicates hydralazine; ISDN, isosorbide dinitrate.

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LV Mass and Fibrosis

There was no change in LV mass in any of the trial arms (Table 3). Similarly, there were no changes in ECV, assessed following the administration of gadolinium, in any of the trial arms. Combination therapy with ISDN+hydral increased the native T1 relaxation time ($P=0.021$).

Other LV Geometric Measures

A reduction in EDV was observed in the ISDN ($P=0.037$) and PB ($P=0.037$) arms (Table 3). This occurred in concert with reductions in stroke volume for ISDN ($P=0.029$) and a trend towards reduced stroke volume in PB ($P=0.054$). In contrast, combination therapy with ISDN+hydral tended to increase EDV ($P=0.052$) and significantly increased stroke volume ($P=0.002$).

Additional Assessments

NT-pro-BNP levels, mitral E/septal e’ ratio, left atrial volume index (Table 4), and the overall summary score for the Kansas City Cardiomyopathy Questionnaire (Table 5) did not change in any of the trial arms. The 6MW distance was unchanged in both the ISDN and PB groups; however, 6MW distance worsened in the ISDN+hydral arm (baseline 343.3 [95% CI, 319.2–367.4]; final 277.0 [95% CI, 242.7–311.4] meters; $P=0.022$).

Sensitivity Analyses

Paired analyses using only data from the baseline and final visits were also performed on select end points (Table 6). The results of these sensitivity analyses were consistent with the findings using the mixed models approach.

Adverse Events

Therapy with ISDN or ISDN+hydral was poorly tolerated, with significantly more patients experiencing adverse events in both active arms as compared with those taking PB (ISDN, n=8 [61.5%]; ISDN+hydral, n=9 [60.0%], PB, n=2 [12.5%]; $P=0.007$ [Table 7]). Common side effects in the active treatment arms were headache, dizziness/lightheadedness, hypotension, and orthostasis.

Discussion

In this randomized pilot trial, we examined the impact of ISDN, ISDN+hydral, and PB on wave reflections, LV remodeling, 6MW distance, NT-pro-BNP, and quality of life. Contrary to our hypothesis, ISDN significantly reduced aortic $Z_c$ and $P_r$ but did
**Echocardiographic Indices of Diastolic Function**

| Marginal | ISDN+Hydral | ISDN | Hydral |
|----------|-------------|------|--------|
| Value    | 3 Months | Final | 3 Months | Final |
| E, cm/s  | 80.4 (78.2–89.0) | 74.6 (71.7–83.2) | 81.8 (78.9–97.2) | 77.4 (71.7–85.4) |
| A, cm/s  | 80.4 (78.9–97.2) | 77.7 (74.3–88.6) | 80.4 (78.0–89.7) | 78.3 (71.7–85.5) |
| E/A ratio | 0.98 (1.01–1.22) | 0.93 (0.91–1.14) | 0.89 (0.87–1.02) | 0.72 (0.66–0.79) |
| Septal e', cm/s | 15.2 (13.8–15.5) | 13.9 (12.3–14.6) | 15.5 (13.6–17.0) | 14.6 (12.3–16.3) |
| E/e' ratio | 11.9 (10.6–15.1) | 11.9 (10.6–15.1) | 11.9 (10.6–15.1) | 11.9 (10.6–15.1) |
| LAVI, mL/m^2 | 34.7 (28.3–40.6) | 34.7 (28.3–40.6) | 34.7 (28.3–40.6) | 34.7 (28.3–40.6) |

Hydral indicates hydralazine; ISDN, isosorbide dinitrate; LAVI, left atrial volume index.

*P* < 0.05 between baseline and the 3-month visit.

**Table 4.**

With LV contraction, a pulse wave is generated that propagates down the arterial tree. When this wave encounters sites of impedance mismatch, such as at bifurcations, a portion of this pulse wave is reflected back towards the heart. Optimally timed wave reflections increase diastolic pressure and coronary perfusion, without exerting pronounced effects in central systolic pressure or left ventricular load. In individuals with increased vessel stiffness, the reflected wave arrives back at the heart earlier, increasing the mid-to-late systolic workload of the left ventricle. This increase in late-systolic load has been shown to induce LV hypertrophy, impair systolic function and diastolic function, and increase myocardial fibrosis. Therapies that reduce RM are associated with regression in LV mass in hypertensive patients, suggesting that RM may be a potential therapeutic target in HFpEF patients, who are generally hypertensive, and exhibit prominent wave reflections.

In this study, ISDN reduced aortic Zc and P1 but did not reduce RM. The venodilating effect of ISDN, which would reduce preload and stroke volume, as well as the effects of this drug on aortic Zc, can explain the reduction in P1, yet the neutral effects on RM bear additional mention. It is possible that chronic administration of organic nitrate led to increased oxidative stress and worsened endothelial dysfunction, reducing nitric oxide bioavailability and mitigating any long-term benefit on wave reflections. This mechanism could be particularly prominent in HFpEF patients, a population in which oxidative stress and decreased nitric oxide bioavailability have been demonstrated. Second, the long-term administration of ISDN could exert hemodynamic effects on multiple arterial segments, with differential impact on RM. ISDN reduced aortic Zc, which may have counteracted the vasodilatory effects on more distal vessels, such as the muscular arteries. Consequently, overall impedance matching may have been unchanged, leading to similar RM. Finally, differences in the vasculature of HFpEF patients may have led to different responses than that which occurs in patients with hypertension or HFrEF.

An unexpected finding of our study was the increase in RM seen after 6 months of ISDN+hydral administration, along with reduced 6MW distances, increased EDV, and increased native T1 relaxation time. Prior work in a small number of HF patients found no change in sodium excretion following 3 days of hydralazine, although renin activity increased. In
Table 5. Kansas City Cardiomyopathy Questionnaire Results

| Marginal Means (95% CI) | ISDN | ISDN+Hydral | Placebo | P Value |
|-------------------------|------|-------------|---------|---------|
|                         | Baseline (n=11) | 3 Months (n=8) | Final (n=6) | Baseline (n=13) | 3 Months (n=8) | Final (n=9) | Placebo Baseline (n=16) | 3 Months (n=8) | Final (n=9) | P Value |
| Physical Limitation Score | 49.6 (41.2–58.0) | 52.1 (42.9–61.3) | 65.9 (55.4–76.4) | 0.09 | 51.1 (42.4–59.7) | 49.4 (38.2–60.6) | 51.3 (41.3–61.3) | 0.97 | 55.5 (44.5–66.5) | 54.8 (39.4–70.1) | 59.0 (44.6–73.3) | 0.91 |
| Symptom Stability Score | 44.9 (31.8–57.9) | 54.3 (39.4–69.1) | 62.1 (44.6–79.6) | 0.35 | 51.1 (39.5–62.7) | 61.4 (46.3–76.5) | 46.7 (33.2–60.1) | 0.39 | 53.6 (44.3–62.9) | 54.3 (41.4–67.3) | 73.0 (60.9–85.1) | 0.06 |
| Total Symptom Score     | 48.1 (38.1–58.1) | 57.0 (45.6–68.3) | 64.1 (50.7–77.5) | 0.23 | 58.2 (49.8–66.7) | 50.2 (39.3–61.1) | 50.1 (40.4–59.8) | 0.43 | 55.8 (44.2–67.5) | 60.0 (43.8–76.1) | 68.7 (53.6–83.8) | 0.46 |
| Self-Efficacy Score      | 67.2 (54.5–79.9) | 84.4 (70.0–98.8) | 78.9 (61.9–95.9) | 0.26 | 70.1 (57.5–82.7) | 58.4 (42.0–74.7) | 39.9* (25.4–54.5) | 0.03 | 62.0 (54.4–69.7) | 85.6* (74.2–96.9) | 78.7* (68.9–88.6) | 0.016 |
| Functional Status Score  | 48.5 (41.6–55.4) | 54.4 (46.5–62.2) | 64.8 (55.5–74.0) | 0.06 | 54.6 (46.7–62.6) | 49.8 (39.4–60.2) | 50.7 (41.5–59.9) | 0.75 | 55.7 (45.5–65.9) | 57.4 (43.1–71.6) | 63.8 (50.5–77.1) | 0.65 |
| Overall Summary Score    | 43.5 (35.3–51.6) | 50.6 (41.3–59.9) | 62.1 (51.1–73.1) | 0.07 | 49.1 (42.4–55.9) | 45.2 (36.4–54.0) | 44.9 (37.1–52.7) | 0.70 | 54.0 (45.3–62.7) | 57.6 (45.5–69.7) | 62.1 (50.8–73.4) | 0.58 |

Hydral indicates hydralazine; ISDN, isosorbide dinitrate.

*P < 0.05 between baseline and the 3-month visit.
†P < 0.05 between baseline and the final visits.
Table 6. Paired Analyses of Select End Points Using Only Data From the Baseline and Final Visits

|                                | ISDN (n=7) | ISDN+Hydral (n=7) | Placebo (n=6) |
|--------------------------------|------------|------------------|--------------|
|                                | Baseline   | Final            | Difference   | P Value   | Baseline | Final            | Difference | P Value |
| iLVEDV, mL/m²                  | 70.4 (14.5)| 59.7 (15.4)      | -10.6 (10.5) | 0.037     | 68.6 (11.4)| 76.6 (12.0)      | 8.0 (8.7)  | 0.052  |
| Hydral (n=7) Placebo (n=6)     |            |                  |              |           | 80.7 (24.2)| 65.9 (14.8)      | -14.8 (12.9)| 0.037  |
|                                |            |                  |              |           | 50.6 (16.5)| 41.4 (10.8)      | -9.2 (8.9) | 0.054  |
| iSV, mL/m²                     | 42.7 (6.8) | 37.2 (8.3)       | -5.5 (5.2)   | 0.029     | 39.6 (4.7) | 48.2 (5.8)       | 8.6 (4.3)  | 0.002  |
|                                |            |                  |              |           | 50.6 (16.5)| 41.4 (10.8)      | -9.2 (8.9) | 0.054  |
| Native T1, ms                  | 1011.0 (19.1)| 1003.7 (39.5)  | -7.33 (42.6) | 0.69      | 1022.6 (47.0)| 1060.7 (56.1)  | 38.1 (32.6)| 0.02   |
|                                |            |                  |              |           | 1013.9 (66.8)| 1013.4 (48.0)  | -0.55 (85.2)| 0.98  |

6MW indicates 6-minute walk distance; Hydral, hydralazine; iLVEDV, indexed left ventricular end-diastolic volume; ISDN, isosorbide dinitrate; iSV, indexed stroke volume; Pb, backward pressure waves; Pf, forward pressure waves; SBP, systolic blood pressure; Zc, characteristic impedance of ascending aorta.
Table 7. AEs in Patients Who Started Study Medications

| No. (%) | ISDN (n=13) | ISDN+Hydral (n=15) | Placebo (n=16) | P Value |
|---------|-------------|-------------------|--------------|---------|
| Any AE  | 8 (61.5)    | 9 (60.0)          | 2 (12.5)     | 0.007   |
| Treatment-related AE | 6 (46.2) | 6 (40.0) | 1 (6.3) |
| Headache | 4 (30.8) | 2 (13.3) | 1 (6.3) |
| GI symptoms | 0 (0) | 1 (6.7) | 0 (0) |
| Dizziness/lightheadedness | 2 (15.4) | 2 (13.3) | 0 (0) |
| Hypotension | 1 (7.7) | 3 (20.0) | 0 (0) |
| Orthostasis | 1 (7.7) | 2 (13.3) | 0 (0) |
| Fatigue | 0 (0) | 1 (6.7) | 0 (0) |
| Reduced renal function | 1 (7.7) | 0 (0) | 0 (0) |
| Discontinued study medications due to related AE | 4 (30.8) | 3 (20.0) | 1 (6.3) |
| Treatment-unrelated AE | 2 (15.4) | 6 (40.0) | 1 (6.3) |
| GI symptoms | 1 (7.7) | 1 (6.7) | 1 (6.3) |
| Reduced renal function | 0 (0) | 0 (0) | 1 (6.3) |
| Retinal hemorrhage | 1 (7.7) | 0 (0) | 0 (0) |
| Bacterial pneumonia | 0 (0) | 1 (6.7) | 0 (0) |
| Atrial fibrillation | 0 (0) | 2 (13.3) | 0 (0) |
| Atypical chest pain | 1 (7.7) | 1 (6.7) | 1 (6.3) |
| Cauda equina syndrome | 0 (0) | 0 (0) | 1 (6.3) |
| Cellulitis | 0 (0) | 1 (6.7) | 0 (0) |
| Rhabdomyolysis | 0 (0) | 0 (0) | 1 (6.3) |

AE indicates adverse event; GI, gastrointestinal; Hydra, hydralazine; ISDN, isosorbide dinitrate.

V-HeFT II (the Vasodilator-Heart Failure Trial), treatment with ISDN+hydral increased norepinephrine levels. More recently, hydralazine has been shown to activate preganglionic sympathetic neurons. Our findings of increased preload (suggesting volume retention), adverse interstitial remodeling, and reduced 6MW distances may be consistent with sympathetic nervous system activation. Other manifestations of sympathetic activation, such as tachycardia, could have been masked by the high utilization of concomitant cardiovascular medications, such as β-blockers.

In our study, we found a significant increase in pregadolinium native T1 time in the ISDN+hydral group, in the absence of an increase in extracellular volume fraction, assessed following the administration of gadolinium. Several explanations may underlie this finding. First, ECV calculations reflect interstitial changes, given its reliance on gadolinium, an extracellular contrast agent. Native T1 signals, however, arise from the entirety of the myocardium, and thus reflect both the intracellular as well as the interstitial spaces. The increased native T1 time, in the absence of a change in ECV, may thus be a reflection of changes occurring at the intracellular level (cardiomyocytes, cardiac fibroblasts, smooth muscle cells, and endothelial cells), including increased edema or cardiomyocyte disarray.

Finally, treatment with ISDN, with or without hydralazine, was poorly tolerated in this population and led to frequent adverse events. Overall, our findings are consistent with those of NEAT-HFpEF (Nitrate’s Effect on Activity Tolerance in Heart Failure with Preserved Ejection Fraction) trial, which showed that isosorbide mononitrate decreased physical activity on accelerometry and led to numerically greater adverse events as compared with PB. While patients in NEAT-HFpEF were predominantly white women, as opposed to our predominantly black male population, the consistency of the findings suggests that the addition of hydralazine to organic nitrate therapy, a useful therapeutic approach in HFrEF, is not a suitable approach in HFpEF. In particular, the increased wave reflections, increased EDV, worsened exercise capacity, and poor tolerability observed in our trial argue against a potential benefit of this drug combination in HFpEF.

Interestingly, in contrast to organic nitrate, inorganic nitrate and nitrite have been shown to reduce wave reflections, improve endothelial function, blunt the exercise-induced rise in pulmonary capillary wedge pressure, and enhance exercise capacity in 2 separate trials of patients with HFpEF. The effects of sustained administration of inorganic nitrate (KNO₃) in HFpEF are currently being examined in a phase Ib trial sponsored by the National Heart, Lung, and Blood Institute (KNO₃CK OUT HFPEF trial, ClinicalTrials.gov: NCT02840799). A trial of inhaled inorganic nitrite in HFpEF is also underway (ClinicalTrials.gov: NCT02713126).

Study Strengths and Limitations

Our study should be interpreted in the context of its strengths and limitations. The strengths of our study include its double-blinded randomized design, the use of state-of-the-art methods for central pressure and flow assessments, hemodynamic modeling, and the noninvasive characterization of myocardial structure and function. Our study also has limitations, mainly related to its small sample size. Despite flexibility in scheduling and compensation for participation, only 27 of 44 (61%) patients who started the study medications completed the study. Unfortunately, the poor tolerability of the study interventions themselves contributed to the increased number of patients who prematurely left the study. Of the 17 patients who withdrew after starting study medications, 8 (47%) withdrew because of side effects...
Conclusions

Despite the small sample size, the high incidence of adverse events and the within-group changes demonstrated in the active arms in our study do not support the use of ISDN, with or without hydralazine, in patients with HFpEF.

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