Nitroxyl (HNO)
A Novel Approach for the Acute Treatment of Heart Failure

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Background—The nitroxyl (HNO) donor, Angeli's salt, exerts positive inotropic, lusitropic, and vasodilator effects in vivo that are cAMP independent. Its clinical usefulness is limited by chemical instability and cogeneration of nitrite which itself has vascular effects. Here, we report on effects of a novel, stable, pure HNO donor (CXL-1020) in isolated myoctyes and intact hearts in experimental models and in patients with heart failure (HF).

Methods and Results—CXL-1020 converts solely to HNO and inactive CXL-1051 with a $t_{1/2}$ of 2 minutes. In adult mouse ventricular myocytes, it dose dependently increased sarcomere shortening by 75% to 210% (50–500 μmol/L), with a ≈30% rise in the peak Ca^{2+} transient only at higher doses. Neither inhibition of protein kinase A nor soluble guanylate cyclase altered this contractile response. Unlike isoproterenol, CXL-1020 was equally effective in myocytes from normal or failing hearts. In anesthetized dogs with coronary microembolization-induced HF, CXL-1020 reduced left ventricular end-diastolic pressure and myocardial oxygen consumption while increasing ejection fraction from 27% to 40% and maximal ventricular power index by 42% (both $P<0.05$). In conscious dogs with tachypacing-induced HF, CXL-1020 increased contractility assessed by end-systolic elastance and provided venoarterial dilation. Heart rate was minimally altered. In patients with systolic HF, CXL-1020 reduced both left and right heart filling pressures and systemic vascular resistance, while increasing cardiac and stroke volume index. Heart rate was unchanged, and arterial pressure declined modestly.

Conclusions—These data show the functional efficacy of a novel pure HNO donor to enhance myocardial function and present first-in-man evidence for its potential usefulness in HF.

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Patients with acute decompensated heart failure (HF) present a complex and often life-threatening clinical syndrome. New therapeutic advances remain scant, and patients are at major risk for recurrent hospitalizations and have a high mortality rate.[1–3] The initial thrust of therapy focuses on decongestion and hemodynamic stabilization, with removal of excess fluid by diuresis or ultrafiltration,[4] and use of arterial and venous dilators to reduce preload and afterload.[3,5] In a substantial number of patients, these approaches prove insufficient or cannot be adequately used because of renal dysfunction and hypotension. In such individuals, inotropes are often considered,[6] although this avenue has been historically limited by difficulties in separating therapeutic benefit from unwanted toxicity. The most commonly used positive inotropic agents are dobutamine or milrinone, but both confer adverse myocardial effects, including tachycardia and arrhythmia linked to cAMP-dependent signaling,[7] and can worsen long-term outcomes.[8,9] Several new strategies are being pursued, including omecamtiv mecarbil, an activator of myosin ATPase,[10,11] and istaroxime that is thought to impact calcium handling.[12] Safe and effective therapies that enhance left ventricular (LV) function and also aid in decongestion remain lacking.

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Nitroxyl (HNO) is a reactive nitrogen species that although related to NO displays many unique biochemical and pharmacological features.[13,14] HNO improves myocardial function by direct positive cAMP-independent lusitropic and inotropic effects and by combined venous and arterial dilation.[15–19] HNO targets selective cysteine residues (negatively charged, or thiolates) resulting in covalent bonding or formation of a reversible disulfide. In myocytes, HNO enhances sarcoplasmic reticular (SR) calcium uptake and release via cysteine modifications on SERCA2a,[19,20] phospholamban,[21,22] and the ryanodine receptor[19] and also improves myofilament calcium sensitivity.[15,23] HNO does not alter L-type calcium channel current or total SR calcium load.[16] Unlike its myocyte effects, vasodilation from HNO has been attributed to soluble guanylate cyclase activation,[24,25] although other pathways remain possible. Importantly, the effects of HNO on the heart are (1) independent of cAMP or cGMP, (2) similar in normal and failing myocardium, (3) minimally impacted by β-adrenergic receptor blockade, and (4) additive with agents stimulating cAMP/protein kinase A pathways (eg, β-receptor agonists), unlike NO.[17]

The compendium of pharmacological effects of HNO donors has suggested potential for treating both congestion and hemodynamic insufficiency in acute decompensated HF. However, major limitations in available HNO donors have impeded progress in the field. Virtually all prior studies have used the inorganic compound Angeli's salt (AS; $Na_2N_2O_3$)[26] that is chemically unstable and thus unsuitable for clinical use. AS also cogenerates nitrite which itself has potent vascular effects.[27] To circumvent these limitations, we developed a novel Piloty's acid cogener, CXL-1020, which nonenzymatically decomposes to produce pure HNO and an inactive organic by-product (CXL-1051). We tested the impact of CXL-1020 on isolated myocyte function and calcium transients, determined its dose-dependent efficacy in vivo in 2 canine models of cardiac failure, and performed the first clinical study of an HNO donor testing proof-of-concept for patients with decompensated HF. The results support the potential usefulness of HNO donors as a novel HF treatment.

Methods

Pharmacology of CLX-1020

CXL-1020 (Cardioxyl Pharmaceuticals, Chapel Hill, NC) was synthesized as a pure HNO donor that chemically decomposes to HNO and an organic by-product (CXL-1051). CXL-1051 has no cardiovascular pharmacological activity and is not metabolized in vivo but rather excreted unchanged in the urine. In PBS buffer, the decay half-times of CXL-1020 and generation of HNO and CXL-1051 measured by reverse phase high-performance liquid chromatography were 1.9, 1.5, and 2.1 minutes, respectively (Figure 1A; conditions for high-performance liquid chromatography analysis provided in Table I in the online-only Data Supplement and quantitation shown in Figure I in the online-only Data Supplement). At high concentrations, HNO rapidly dimerizes in aqueous solution to HON-NOH which decomposes to nitrous oxide (N_2O) and water. Thus, in the test tube, HNO generation is measurable by quantifying N_2O by gas chromatography headspace analysis. The disappearance of CXL-1020 and appearance of N_2O and CXL-1051 were highly correlated (Figure 1A), and importantly, 100% degradation of CXL-1020 yielded 100% appearance of N_2O and CXL-1051, confirming CXL-1020 did not generate other NO species such as NO or nitrite. Quantitative conversion of CXL-1020 to CXL-1051 was also documented in EDTA-treated whole human blood (Figure 1B), with $t_{1/2}$ for loss of CXL-1020 and formation of CXL-1051 being 2 minutes. CXL-1020 is stable (>95%) in aqueous solution at pH <4.5 for ≥24 hours and soluble to ≈1 mg/mL in water for injection, 5% dextrose, and 0.9% saline, and 100 μmol/L citrate pH 4.0. Higher concentrations (≤30 mg/mL) were achieved by formulation with a β-cyclodextrin (Captisol).

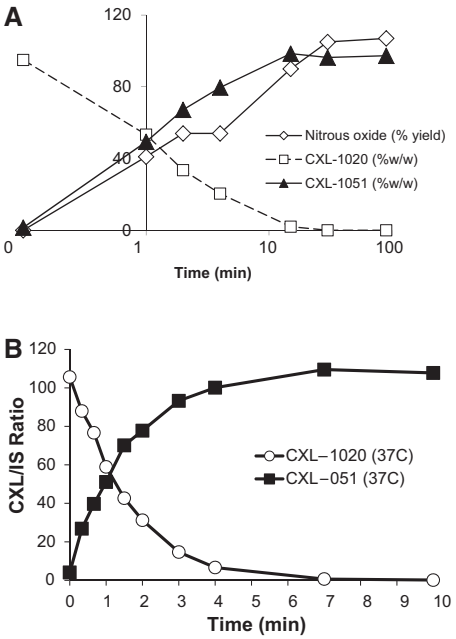

Figure 1. Pharmacological decomposition of CXL-1020. **A**, Decomposition of CXL-1020 in aqueous solution into nitroxyl (HNO; measured by nitrous oxide) and CXL-1051. Conversion is rapid and virtually complete by 15 minutes, with stoichiometry confirming pure generation of HNO and CLX-1051 in equal parts. **B**, Decomposition of CXL-1020 in human whole blood shows similar rapid pharmacokinetics. IS indicates internal standard. See Methods in the online-only Data Supplement.

In Vitro Myocyte Studies

Adult LV cardiomyocytes were isolated from male 3- to 6-month-old C57Bl/6 mice (Jackson Laboratory, Bar Harbor, ME) with either normal or failing hearts (latter induced by 9-week transverse aortic constriction).[28] Details are provided in Methods in the online-only Data Supplement. Cells were studied at room temperature, superfused in Tyrode's solution, and stimulated at 0.5 Hz. Sarcomere shortening and twitch kinetics were measured by inverted fluorescence microscopy (Ellipse TE2000, Nikon Inc) using Fourier-image analysis (MyoCam, IonOptix, MA). Cells were preincubated with Fura-2/AM (Molecular Probes, 3 μmol/L for 10 minutes, de-esterification 20 minutes) to measure whole-cell Ca^{2+} transients. Cells were then exposed to CXL-1020 (50–500 μmol/L), prepared from a 100 mmol/L stock solution in 100% DMSO (final concentration of DMSO of 0.05%–0.5%).

In Vivo Canine Studies

Two canine models of cardiac failure were studied. All studies followed procedures approved by the respective Institutional Animal Care and Use Committee of the Johns Hopkins Medical Institutions or Henry Ford Hospital.

Group A dogs had ischemic cardiomyopathy generated by serial coronary microembolization[29] (Methods in the online-only Data Supplement). An initial dose-finding study (n=3; CXL-1020, 3–100 μg/kg per minute×40 minutes) identified 2 doses (n=6; 3 or 10 μg/kg per minute×4 hours) for subsequent hemodynamic analysis. CXL-1020 was mixed with 7% Captisol in sterile water at pH=4 (vehicle), the latter then used as vehicle control. Data were obtained under general anesthesia induced by intravenous hydromorphone (0.22

mg/kg) and diazepam (0.17 mg/kg) and maintained with 1% to 2% isoflurane. Cardiac function was assessed by micromanometer arterial and LV pressures, right heart catheterization pressures, contrast ventriculography (LV volumes) and echo Doppler cardiography, as previously reported.[29] LV peak power index ($P_{max}I$) was estimated by (peak aortic flow velocity×peak systolic pressure)/EDV^2, where EDV is the end-diastolic volume.[30] Diastolic function was assessed by deceleration time of mitral inflow velocity and ratio of early to late filling time integrals (E_i/A_i). Myocardial oxygen consumption was assessed at baseline and after 4 hours as previously described[31] (details in Methods in the online-only Data Supplement). At the highest dose, blood samples were obtained to determine N-terminal pro–brain natriuretic peptide, pro–atrial natriuretic peptide, and troponin I by ELISA assay per manufacturer's instructions (Methods in the online-only Data Supplement).

A separate group of animals (n=6) were subjected to programmed ventricular stimulation after receiving 5 μg/kg per minute CXL-1020×40 minutes or vehicle. Each study was terminated when extrastimuli provoked sustained monomorphic ventricular tachycardia for >30 seconds or ventricular fibrillation.

In group B dogs, HF was induced by 3-week tachypacing. Conscious dogs were chronically instrumented to obtain LV pressure–volume relations, including an LV micromanometer (P22; Konigsberg Instruments, Pasadena, CA), right atrial and descending aortic catheters, 3 pairs of orthogonal endocardial sonomicrometers to assess LV volume, and inferior vena caval cuff occluder.[17] Epicardial pacing leads sutured to the LV free wall were connected to an implanted pacemaker (Spectrax, Medtronics, Minneapolis, MN). Data were recorded in conscious animals both at initial baseline when the heart was normal and after induction of HF. Pressure–volume relations were obtained and used to assess end-systolic elastance (Ees) and preload-recruitable stroke measures of contractile function, and steady-state data were used to assess chamber volumes and pressures and isovolumic relaxation time constant.[32] Baseline data were obtained during vehicle infusion, and then CXL-1020+vehicle was administered at doses ranging from 3 to 100 μg/kg per minute. Data were digitally recorded (200 Hz) at each dose after reaching a steady-state response (≈10 minutes).

Human HF Studies

A prior phase 1-2a pilot study in patients with stable HF (Clinicaltrials.gov NCT01092325) identified 4-hour exposure to CXL-1020 at 1 to 30 μg/kg per minute as safe and potentially active.[23] The present study (Clinicaltrials.gov NCT010960430) examined the hemodynamic effects and safety of CXL-1020 at doses of 1 to 20 μg/kg per minute in patients hospitalized for hemodynamic assessment of HF before transplantation or for treatment of decompensated HF requiring hemodynamic monitoring. Institutional review board approval was obtained at each institution involved with the trial, pursuant to federal guidelines, and all subjects provided informed consent. Measurements were obtained within 72 hours of hospitalization. Inclusion criteria required a mean cardiac index ≤2.5 L/min and a mean pulmonary capillary wedge pressure or pulmonary artery diastolic pressure >20 mm Hg at baseline, based on 3 consecutive cardiac index and pulmonary capillary wedge pressure measurements within 10% agreement and measured in the hour preceding drug administration. Baseline diuretic and oral vasodilator therapy was withheld for ≥3 hours before baseline recordings, and no parenteral hemodynamically active agents were allowed within 12 hours of baseline measurements. Patients with a heart rate (HR) <50 or ≥100 beats per minute, a systolic blood pressure of <100 or >150 mm Hg, or a diastolic blood pressure of >95 mm Hg at baseline before randomization were excluded. Also excluded were patients with atrial fibrillation without adequate rate control, or with evidence of clinically significant nonsustained ventricular tachycardia (10 beats or at a rate >120 beats per minute) in the preceding 12 hours. CXL-1020 was administered intravenously using a placebo-controlled (4:1 active-to-placebo randomization ratio) 6-hour forced titration design, with uptitration at 2-hour intervals. An overall dose range of 1 to 20 μg/kg per minute was studied using overlapping dose ranges in 2 cohorts (cohort 1=1, 3 and 10 μg/kg per minute; cohort 2=3, 10 and 20 μg/kg per minute).

Statistical Analysis

For myocyte studies, data were analyzed using paired responses for a given set of cells that were exposed to a particular dose of test drug. Because these differences were often not normally distributed, we tested the null hypothesis (percent change=0) using a Wilcoxon signed-rank test. For intact canine studies group A, within group comparisons were made using repeated measures ANOVA with α set at 0.05. If significance was attained, then pairwise comparisons were made using the Student-Newman-Keuls test with $P<0.05$ considered significant. For group B, parameters were assessed by repeated measures ANCOVA, with a Tukey test for multiple comparisons, and normal versus failing data compared by nonparametric (Wilcoxon) test. For clinical studies, change from baseline of a hemodynamic parameter measured at a given time point following drug infusion (or placebo) was determined, and these differences were then compared by *t* test to to determine whether drug response differed from placebo (placebo-corrected response). Data are reported as mean±SEM.

Results

CXL-1020 Improves Myocyte Systolic and Diastolic Function

Figure 2A shows example sarcomere length and Ca^{2+} tracings before and after exposure to CXL-1020 (50 μmol/L). Sarcomere shortening rose substantially in a dose-dependent manner, reaching ≈210% over baseline at 500 μmol/L. Peak Ca^{2+} transients rose more modestly (+30% at the highest dose), displaying little change at lower doses (Figure 2B). Diastolic Ca^{2+} was little altered (≤3%) at any of the doses (data not shown). Systolic functional changes were accompanied by a faster rate of sarcomere relengthening and shortening of the Ca^{2+} transient decay (Figure 2C). The decomposition product CXL-1051 had no direct impact on the cells (Figure IIA in the online-only Data Supplement).

To test whether functional effects of CXL-1020 required protein kinase A or cGMP-dependent signaling, cells were coincubated with either Rp-cAMPs (protein kinase A inhibitor, 100 μmol/L×30 minutes) or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μmol/L×30 minutes) to block soluble guanylate cyclase (Figure 2D). Neither intervention impacted CXL-1020 modulation of sarcomere shortening. Rp-cAMPs or ODQ incubation alone had no effects on basal cell shortening at the concentrations studied (percent shortening 3.4±0.3% with Rp-cAMPs, 3.4±0.4% with ODQ, both $P>0.22$ versus 3.0±0.19% for non-treated cells). As first demonstrated with AS,[18] CXL-1020 mediated contractility was also redox sensitive, being suppressed by preincubating cells with n-acetyl cysteine (5 mmol/L; Figure IIB in the online-only Data Supplement).

We next tested whether CXL-1020 influences myocyte contractility in cells from failing hearts. In contrast to the blunted response to the β-adrenergic agonist isoproterenol (2.5 nmol/L) in failing cells, CXL-1020 induced changes similar to those in normal cells (Figure 2E). In controls, whole-cell Ca^{2+} transients rose (+21±9% and +21±5%) and declined faster (−13±5% and −11±2%) similarly with stimulation by isoproterenol or CXL-1020 (each $P<0.05$ versus baseline). However, in failing cells, the isoproterenol Ca^{2+} response was one third that with CXL-1020, and although the decay rate of the Ca^{2+} transient was unaltered by isoproterenol, it shortened −15±5% with CXL-1020. These data support independence of CXL-1020 effects from cAMP/protein kinase A-dependent inotropy or lusitropy that are blunted in failing myocytes.

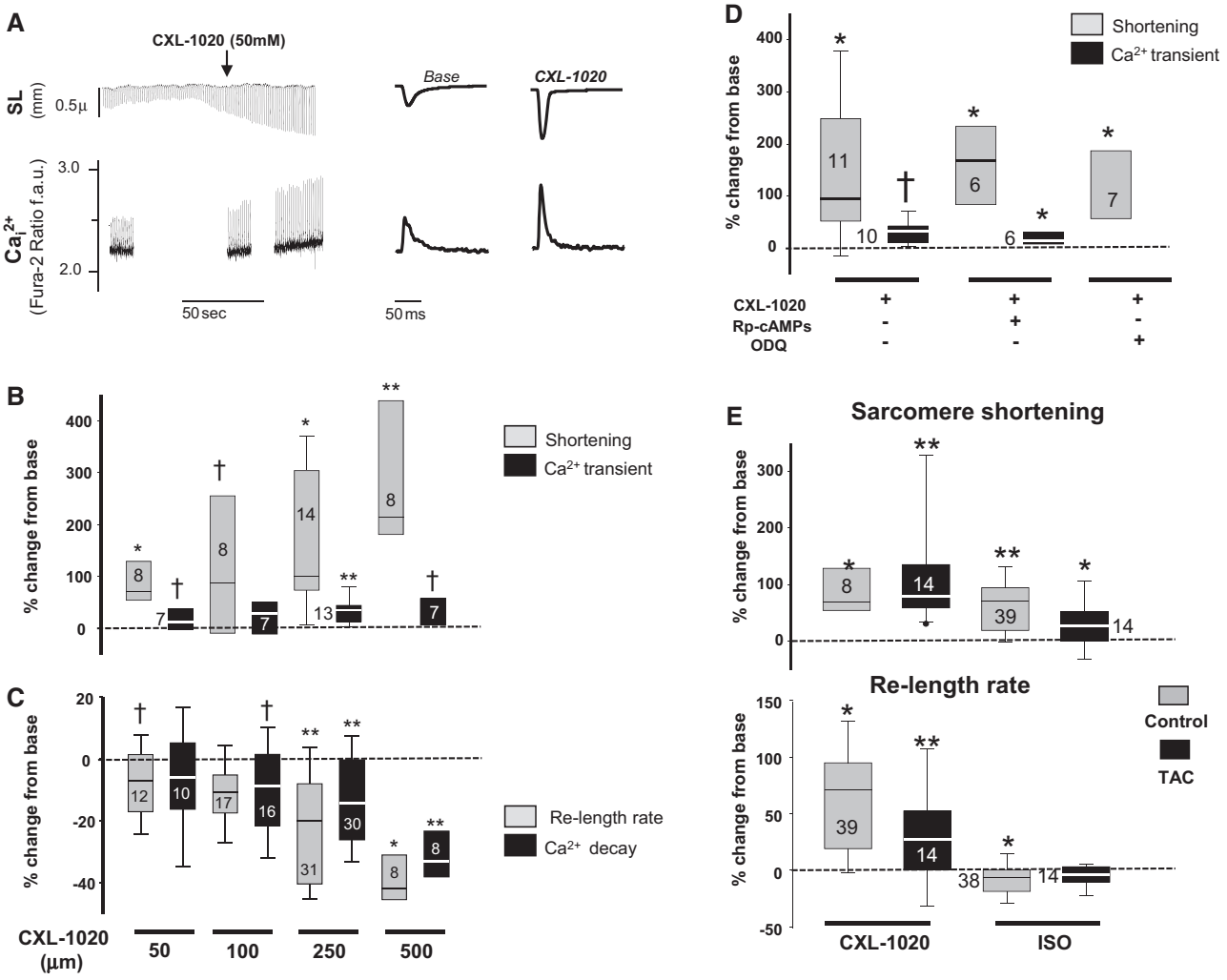

Figure 2. Influence of CXL-1020 on isolated cardiac myocytes from normal and failing heart. **A**, Isolated myocyte sarcomere length (SL, upper tracing) and calcium transients (Ca^{2+}, lower tracing) after exposure to CLX-1020 (**left**). There is a marked rise in sarcomere shortening (SS) and a rise in the peak Ca^{2+} transient, as well as acceleration of the time for relengthening and calcium decline. **B**, Box plots show percent change in SS and peak Ca^{2+} transient relative to baseline with incremental CXL-1020 dose. Only 1 dose was tested per myocyte; the sample size at each dose is provided in the plots. **C**, Percent reduction in sarcomere relengthening and calcium decay time. **D**, Percent change in SS and peak Ca^{2+} transient following CXL-1020 with or without coinhibition of protein kinase A (Rp-cAMPs) or soluble guanylate cyclase (ODQ). **E**, Percent change in SS and relengthening rate in myocytes isolated from control or failing hearts that are then exposed to either isoproterenol (ISO, 2.5 nmol/L) or CXL-1020 (50 µmol/L). †P<0.05; *P<0.01; **P≤0.001 vs respective (pre–CXL-1020, or ISO) baseline, by Wilcoxon signed-rank test. TAC indicates trans-aortic constriction.

In Vivo Effects of CXL-1020 in Canines With Ischemic Cardiomyopathy (Group A)

We next assessed integrative cardiovascular effects of a 4-hour CXL-1020 infusion at 3 or 10 µg/kg per minute (doses derived from preliminary dose-ranging study). Compared with vehicle control, LV end-diastolic volumes declined by ≈15% and ejection fraction rose from 27% to 40% (P<0.05) at the higher CXL-1020 dose (Figure 3). Arterial blood pressures declined slightly during the course of the procedure. They fell 8 to 9 mm Hg more (diastolic and mean) at 3 µg/kg per minute but were unaltered from control at higher doses (Table). End-systolic volume, end-diastolic pressure, and systemic vascular resistance (SVR) all declined. HR was unchanged, and systolic contractility indexed by maximal power index[30] rose 42±2% (P<0.001; Table). Early diastolic function reflected by E_i/A_i filling ratio (Figure 3) and E-wave deceleration time improved, and CXL-1020 also reduced plasma N-terminal pro–brain natriuretic peptide and pro–atrial natriuretic peptide (Table). Plasma troponin I was unchanged.

In Vivo Effects of CXL-1020 in Conscious Canines Using Pressure–Volume Loop Analysis (Group B)

To more directly test whether CXL-1020 enhanced in vivo contractility, pressure–volume analysis was performed in conscious dogs before and after the induction of HF attributable to tachypacing. HF was more severe in group B than group A animals (Table II in the online-only Data Supplement). In failing hearts, CXL-1020 (3–100 µg/kg per minute) lowered end-systolic pressure and volume, and end-diastolic pressure (declined nearly 30%) in a dose-dependent manner, whereas HR was little altered (Figure 4A). Both SVR and time constant of relaxation declined, whereas contractile function indexed by load-insensitive Ees increased. Figure 4B displays example pressure–volume relations before and after CXL-1020, showing a rise in Ees.

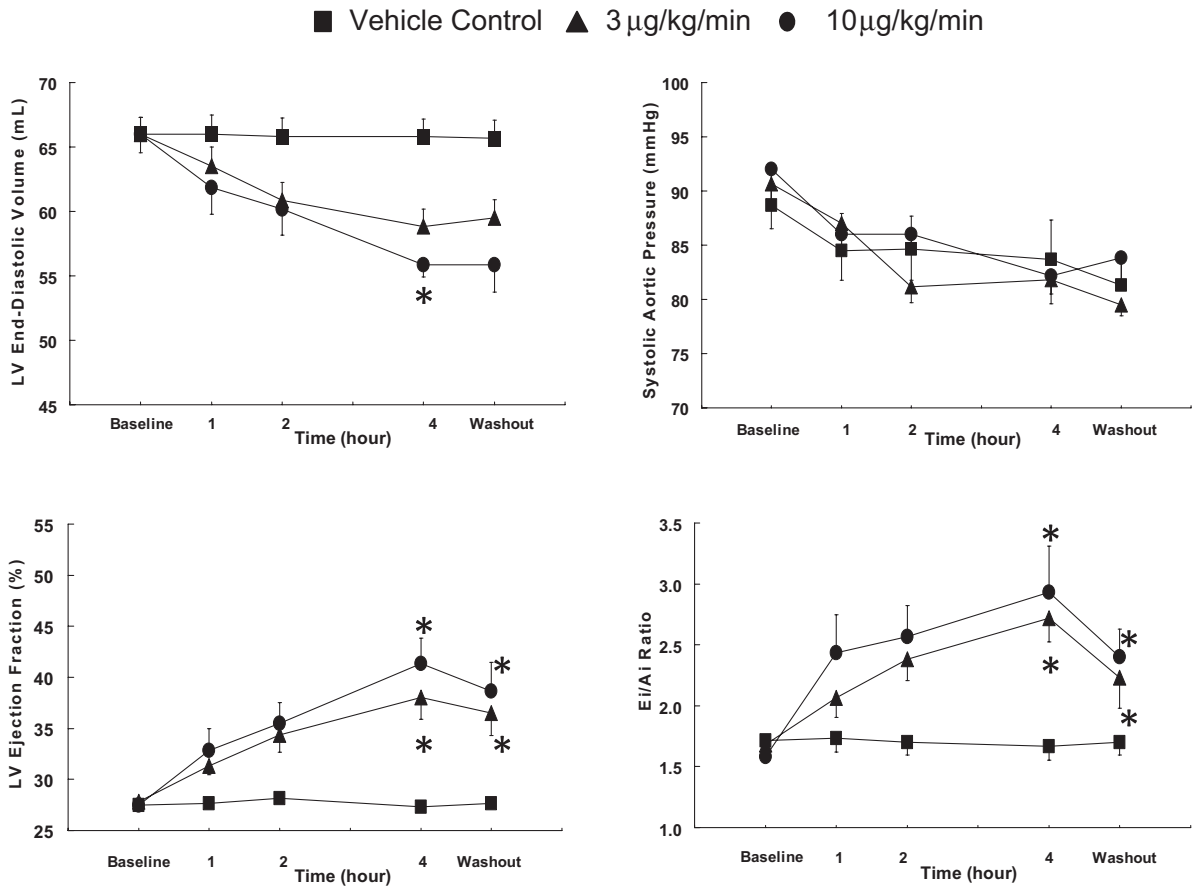

Figure 3. Influence of CXL-1020 in anesthetized dogs during 4-hour infusion. Left ventricular (LV) and systemic hemodynamics at either 3 or 10 μg/kg per minute CXL-1020, with 1 hour of washout (n=6). E_i/A_i indicates ratio of early to late (atrial) mitral inflow. *P<0.05 vs vehicle control by multiple comparisons test (Student-Newman-Keuls) following repeated measures ANOVA.

Many of these responses were also observed when CXL-1020 was administered to the dogs in the control state before inducing HF (Table III in the online-only Data Supplement); however, there were some differences. At the maximal CXL-1020 dose, there was a greater percent decline in preload volume (end-diastolic volume) yet less reduction of SVR in the normal versus HF state (Figure 4C), with ejection fraction consequently rising more in dogs with HF. A similar disparity has been observed with AS.[17,18] HR tended to rise in controls likely reflecting a baroreflex response, but declined slightly in HF dogs. The percent change in Ees was similar in both conditions.

Electrophysiology and CXL-1020

Neither canine model revealed electrophysiological instability in association with CXL-1020 infusion. There were no changes in QTc interval (Figure III in the online-only Data Supplement). In animals subjected to programmed ventricular stimulation, CXL-1020 did not alter the threshold for inducing ventricular tachycardia or fibrillation, or impede cardioversion when either were induced (Table IV in the online-only Data Supplement).

Hemodynamic Effects of CXL-1020 in Patients With Decompensated HF

The demographics, comorbidities, and medications of the 31 study patients are provided in Table V in the online-only Data Supplement. Subject age, sex, HF pathogenesis, and function class were similar between the treatment groups. Baseline hemodynamics (Table VI in the online-only Data Supplement) demonstrated severe systolic HF with a depressed cardiac index (1.9–2.2 L/m per meter squared) and elevated right and left heart filling pressures (15–17 and 25–30 mm Hg, respectively). CXL-1020 was infused at rates of 1 to 20 μg/kg per minute in a placebo-controlled, forced titration protocol in which 2 patient groups received overlapping dose ranges (low-dose group [n=12]=1, 3, and 10 μg/kg per minute; high-dose group [n=12]=3, 10, and 20 μg/kg per minute) and matching placebo (n=7).

HR was unchanged at all doses of CXL-1020, and there were no statistically significant changes in any hemodynamic measure at 1 or 3 μg/kg per minute. Hemodynamic effects of CXL-1020 infusion at 10 and 20 μg/kg per minute are shown in Figure 5. At 10 μg/kg per minute, pulmonary artery diastolic and mean arterial pressure declined. At 20 μg/kg per minute, pulmonary capillary wedge pressure also declined, accompanied by increased cardiac index and stroke volume index, whereas the mean arterial pressure change was insignificant. SVR tended to fall modestly at both 10 and 20 μg/kg per minute doses, reaching significance at the higher dose, whereas right atrial pressure fell significantly at both doses.

CXL-1020 was also evaluated in several echocardiography cohorts (online-only Data Supplement) at doses ≤20 μg/kg per /minute. After 6 hours of infusion, mean arterial pressure was unchanged at any dose; however, at the highest dose, HR

Table. Hemodynamic Variables and Plasma Biomarkers During 4-Hour Infusion of CXL-1020 in Dogs With Ischemic Cardiomyopathy

	Vehicle Control (n=6)			3 μg/kg per min			10 μg/kg per min		
	Baseline	4 h	Washout	Baseline	4 h	Washout	Baseline	4 h	Washout
Heart rate, beats/min	75±4	69±1	71±3	74±2	75±5	65±1	74±1	77±2	75±5
Systolic AoP, mm Hg	89±3	84±4*	81±2	91±2	82±1	80±1	92±6	82±3	84±2
Diastolic AoP, mm Hg	62±3	55±3	57±4	64±2	55±2*	53±1*	64±4	50±1*	53±1
Mean AoP, mm Hg	75±3	71±4	68±3	76±2	68±2*	66±1*	77±5	63±2*	65±2*
LVEDP, mm Hg	13.7±0.8	15.3±0.7	12.3±1.0	14.5±0.7	13.3±0.3	13.8±0.7	13.8±0.3	10.8±0.9*	11.7±1.5
LVESV, mL	47.8±1.0	47.8±1.0	47.7±1.1	47.7±1.1	36.5±2.1*	37.8±2.4*	48.0±1.2	32.8±1.9*	34.3±2.5*
LV EDV, mL	66.0±1.3	65.8.0±1.3	65.7±1.4	66.0±1.3	58.8±2.1*	59.5±2.2*	66.0±1.5	55.8±0.9*	55.8±2.1*
LV FAS, %	25.0±0.4	25.3±0.7	25.2±0.7	25.5±0.3	35.8±2.1*	33.5±2.3*	24.8±0.3	38.8±2.2*	35.3±2.8*
Stroke volume, mL	18.2±0.3	18.0±0.4	18.0±0.4	18.3±0.3	22.3±1.1*	21.7±1.0*	18.0±0.4	23.0±1.3*	21.5±1.5*
SVR, dynes·s·cm^{-5}	4476±328	4640±405	4283±200	4502±142	3335±247*	3793±234*	4642±236	2904±138*	3342±311*
Deceleration time, ms	93.2±2.5	96.3±3.0	96.7±3.6	94.8±3.8	119.8±5.3*	103.3±2.3*	97.3±4.0	128.0±7.5*	121.3±6.9*
Peak power index, (mm Hg/s×mL)×100	184±12	171±10	169±9	192±11	245±19*	212±14	180±14	262±16*	250±20*
MVO_2, μmol/min	…	…	…	…	…	…	102.5±6.3	71.5±14.5*	…
nt-proBNP, fmol/mL	165±12	164±13	…	164±15	133±11*	…	166±13	93±16*	…
Pro-ANP, pmol/mL	0.56±0.06	0.57±0.06	…	0.57±0.07	0.45±0.05*	…	0.57±0.06	0.31±0.05*	…
TnI, ng/mL	0.53±0.03	0.56±0.03	…	0.56±0.03	0.36±0.03*	…	0.58±0.02	0.28±0.02*	…

AoP indicates aortic pressure; EDP, end-diastolic pressure; EDV, end-diastolic volume; ESV, end-systolic volume; FAS, fractional area of shortening; LV, left ventricular; MVO_2, myocardial oxygen consumption; nt-proBNP, N-terminal pro–brain natriuretic peptide; pro-ANP, pro–atrial natriuretic peptide; SVR, systemic vascular resistance; and TnI, troponin I.

*P<0.05 vs baseline.

declined from baseline relative to placebo (P<0.01). There were nonsignificant trends for decreases in LV end-diastolic and end-systolic volumes, an increase in ejection fraction, and increase in stroke volume (Figure IV in the online-only Data Supplement).

Safety and Tolerability of CXL-1020 in Patients With HF

CXL-1020 was well tolerated with few apparent side effects. Both adverse and significant adverse events for the study are provided in Table VII in the online-only Data Supplement. Drug treatment was not terminated for adverse experiences in any patient in this study. There were no adverse trends in routine laboratory parameters for hematology, chemistry, or urinalysis.

Discussion

We report on a novel pure HNO donor, CXL-1020, and demonstrate direct positive inotropic and lusitropic effects in cardiac myocytes from normal and failing hearts, and positive contractile and lusitropic effects and mild vasodilatory effects in failing canine hearts in vivo. Importantly, we translate these findings for the first time to the clinic, finding CXL-1020 enhances cardiac performance while unloading the LV in patients with decompensated systolic HF. CXL-1020 effects were stable during infusion periods ranging from 4 to 6 hours and did not alter HR or induce arrhythmia. This first cell-to-human evaluation of a pure HNO donor suggests the potential efficacy and usefulness of this pharmacological approach to improve the function of the failing heart.

Despite being discovered more than a century ago,[26] AS and the chemistry and physiological role of HNO have only recently received attention. HNO's chemical cousin, NO, has been far more studied, and its signaling roles mediated by chemical modification of cysteines (S-nitrosylation)[33] and activation of soluble guanylate cyclase[34] are widely appreciated. Nonetheless, the 2 molecules are chemically and physiologically distinct, and they do not interconvert under normal physiological conditions. Although the chemistry of HNO also involves post-translational modifications of selective reduced cysteines (thiolates), those modifications result in either a single modified residue (sulfinamide) or induction of a reversible disulfide between neighboring cysteines.[13,21,23] Although NO synthases can produce HNO under conditions of oxidative stress, the endogenous production of HNO remains the subject of considerable speculation and debate. The lack of a bioassay has prevented definitive elucidation of this question. However, there is a growing body of data regarding physiological/pharmacological effects of HNO donors, and this has spawned considerable interest in understanding its biochemistry and potential therapeutic potential.

Paolocci et al[18] originally reported that AS augmented cardiac contractility and relaxation in a cAMP-independent manner. This study also first suggested a link between HNO and secretion of the neuropeptide calcitonin gene–related peptide (CGRP). However, CGRP signaling is coupled to cAMP stimulation, and in subsequent studies, we showed vivo modulation of contractility by CGRP depended on local sympathetic activation rather than a direct effect on cardiomyocytes.[35] Furthermore, the inotropic response to CGRP was markedly blunted in the failing heart,[35] consistent with downregulation of sympathetic stimulation responses in this syndrome. Because AS had direct activity on cardiomyocytes and its impact was not blunted in the failing heart,[17] an alternative to a CGRP mechanism was sought.

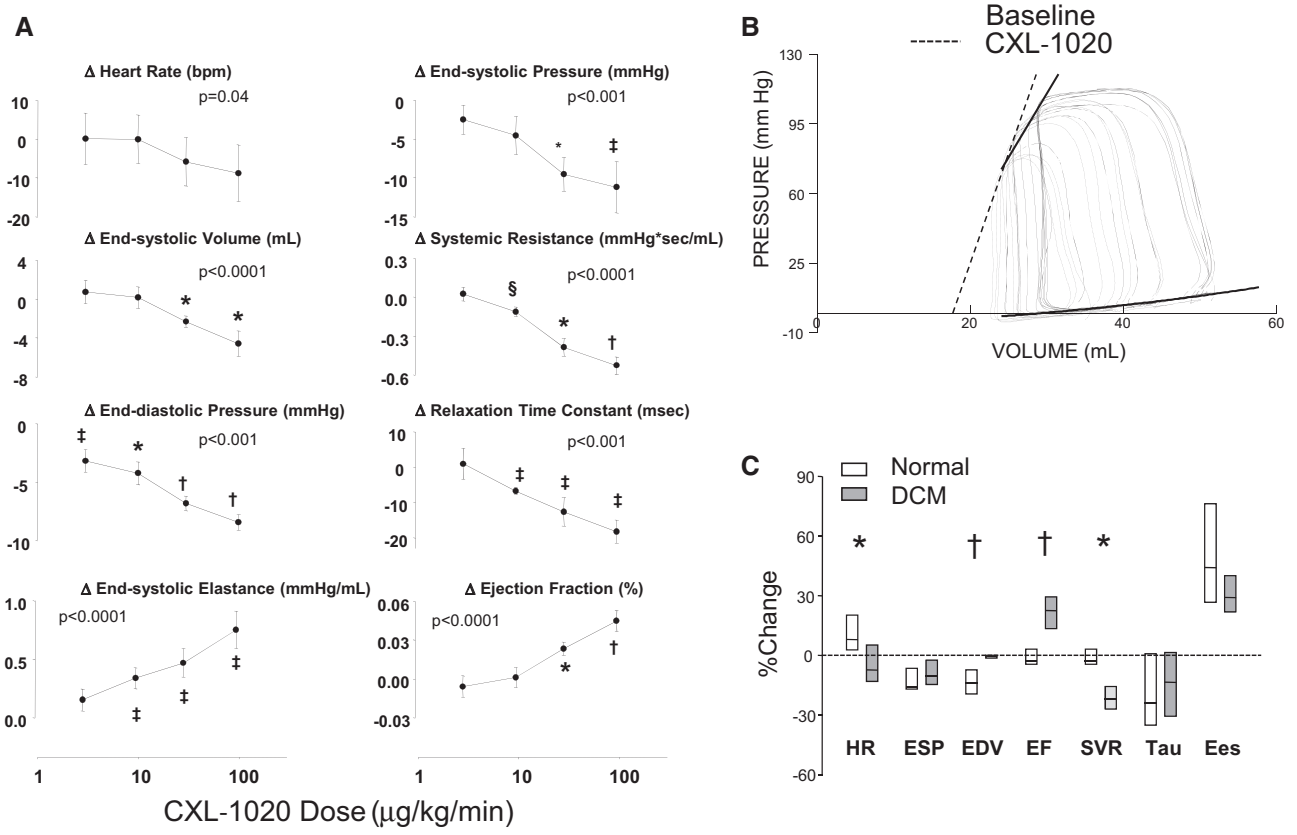

Figure 4. Influence of CXL-1020 in conscious heart failure dogs. **A**, Absolute change in hemodynamic parameters in group B (conscious) heart failure dogs as a function of increasing CXL-1020 dose. *P* values in each plot are for a repeated measures ANCOVA (with drug dose as the continuous variable, n=5). Post hoc multiple comparisons test for dose response vs baseline: **P*<0.005; †*P*<0.001; ‡*P*≤0.01; §*P*=0.02. **B**, Example pressure–volume loops at baseline and after CXL-1020 infusion, showing an increase in the slope of the end-systolic pressure–volume relationship (solid line is control, dashed after CXL-1020). **C**, Box plot for percent change in hemodynamic parameters before and after 100 mg/kg per minute CXL-1020 in normal dogs and the same dogs after inducing heart failure. **P*<0.05, †*P*<0.01 between groups (n=5 per group, Kruskal-Wallis used to test for effect of heart failure on the response). bpm indicates beats per minute; DCM, dilated cardiomyopathy; HR, heart rate; EDV, end-diastolic volume; Ees, end-systolic elastance; EF, ejection fraction; ESP, end-systolic pressure; and SVR, systemic vascular resistance.

More direct mechanistic insights followed the discovery that AS directly improves Ca^{2+} uptake and release from the SR in a manner independent of cAMP or cGMP generation. This cellular behavior was also different from that induced by the NO donor, DEA/NO. Importantly, HNO does not alter L-type calcium channel current[16] or augment SR calcium load,[19] in contrast to agents that generate inotropy by elevating cAMP. Subsequent work revealed a direct impact on enhancing myofilament calcium sensitivity.[15] More recent studies have begun identifying the molecular targets of HNO that could underlie these myocardial effects. Glutathiolation at cysteine 674 has been proposed to link AS inotropy to enhanced SERCA2a activity, and formation of an internal disulfide in phospholamban by AS was shown to enhance SR Ca^{2+} uptake as well.[21] Recently, Sivakumaran et al[22] showed phospholamban (PLN) is required to observe HNO augmentation of both inotropy and Ca^{2+} transients and enhance SR Ca^{2+} uptake and Ca^{2+}-dependent SERCA2a conformational flexibility. This was achieved by stabilizing PLN in an oligomeric disulfide bond-dependent configuration, decreasing the amount of free monomeric (inhibitory) PLN. HNO-induced disulfide links between actin-tropomyosin and myosin heavy chain and myosin light chain have been linked to increased myofilament Ca^{2+} sensitivity.[23] Vascular studies have reported vasodilation attributed to activation of soluble guanylate cyclase and voltage-gated potassium channels.[25] However, it remains possible that smooth muscle SR calcium cycling is involved, and the potential role of NO_2^- cogenerated by AS decomposition remains unresolved.

The present study of CXL-1020 addresses a number of prior critical limitations of AS research. First, it isolates the response to HNO alone, excluding potential effects of the nitrite released by AS. Second, it enables studies of sustained exposure (eg, 4–6 hours), whereas AS instability made such infusion experiments difficult to impossible. The findings with CXL-1020 at both cellular and intact organ levels, in mammalian species ranging from mouse to man, are remarkably compatible with prior data with AS. We observed modest vasodilator effects from HNO that contributed to the integrative functional improvement (eg, stroke volume, ejection fraction) observed. However, using measures that were less load dependent, such as peak power index or Ees, we showed significant increases in contractile function from CXL-1020. The decline in arterial pressure at high doses indicates a direct vasodilator impact of HNO, though CXL-1020 did not either change HR or even result in a slight decline at these doses, likely a result of improved contractile performance. As with AS, the onset

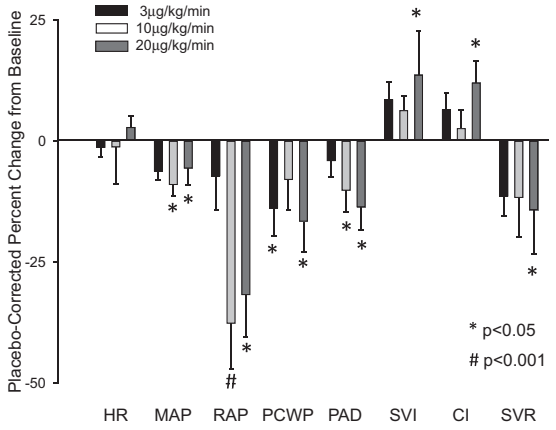

Figure 5. Hemodynamic effects of CXL-1020 in patients with symptomatic heart failure. Effects of CXL-1020 at 3, 10, or 20 µg/kg per minute on heart rate (HR), mean arterial pressure (MAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), stroke volume index (SVI), cardiac index (CI), and systemic vascular resistance (SVR) in 12 patients with symptomatic heart failure (high-dose titration group described in Methods). Data show the mean percent change±SEM at each dose minus the response observed in the placebo group at the same time point (eg, ie, corrected to placebo). *P<0.05 vs baseline, #P<0.005 vs baseline. PAD indicates pulmonary artery diastolic pressure.

of hemodynamic effects with CXL-1020 was rapid, although recovery was somewhat slower. Last, our data show that sustained intravenous administration of a pure HNO donor is not arrhythmogenic. The ischemic HF model (group A) displays easily inducible malignant ventricular arrhythmias,[36] and rate, rhythm, QT interval were not altered by CXL-1020.

Our clinical data for CXL-1020 corroborate the experimental animal data, providing the first-in-man demonstration of the hemodynamic effects of an HNO donor. At the doses and administration studied, CXL-1020 was found to be well tolerated, and a decline in diastolic filling pressures, modest fall in SVR, and rise in cardiac output resulting from increased stroke volume with no change in HR were consistent with inotropic and vasodilator actions observed in dogs, and positive inotropy measured in myocytes.

The clinical study also identified a threshold dose of CXL-1020 for hemodynamic effects at 10 to 20 µg/kg per /minute. Because this dose range did not induce maximal responses in dogs, higher or more prolonged doses were considered. However, in a subsequent longer duration (12–24 hours, at a dose of 20 µg/kg per /minute) study, CXL-1020 was found to produce an inflammatory irritation at the intravenous insertion site. Based on this, it was not thought a viable candidate for further development as a human therapeutic. However, the results with CXL-1020, AS (and other novel HNO donors) confirm that the hemodynamic effects of HNO are a class phenomenon independent of the donor. Second-generation HNO donors have since been developed that abrogate the venous irritation experienced with CXL-1020, while preserving the full spectrum of HNO's hemodynamic effects in animal models, thus providing a viable option for further investigation of the HNO class.

In conclusion, we show that a novel pure HNO donor enhances cardiac systolic function while reducing arterial and venous tone and without increasing HR. Direct contractile enhancement from CXL-1020 is observed in isolated myocytes and experimental hearts in vivo and supported by improved systolic function in humans with congestive HF. The combination of effects differentiates HNO donors from other classes of inotropes or inodilators and provides a strong rationale for continuing studies to develop donors with optimized pharmacological and clinical efficacy for the treatment of congestive HF.

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Disclosures

Dr Sabbah received research grants from and is a consultant to Cardioxyl Pharmaceuticals. Dr Colucci is a consultant to Cardioxyl Pharmaceuticals. Drs Kass and Paolocci are cofounders and are consultants to Cardioxyl Pharmaceuticals. D. Cowart is an employee and Dr Mazhari a prior employee of Cardioxyl Pharmaceuticals. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Nitroxyl is a reactive nitrogen species that interacts with proteins to alter their activity and function, though its chemistry and corresponding pharmacology differ strikingly from the closely related NO. Prior studies using the predominantly studied and >100-year-old nitroxyl donor compound, Angeli's salt, revealed both positive effects on contractility and relaxation, as well as in vivo venous and arterial vasodilation. However, the instability of Angeli's salt and its cogeneration of nitrite, which itself has potent vasodilator effects, limited clinical translation. To circumvent these limitations, we developed a novel pure nitroxyl donor (CXL-1020) which is stable at room temperature. We show that CXL-1020 enhances contractility and relaxation in isolated mouse myocytes and in intact canine models of cardiac failure. CXL-1020 effects occur without engaging the protein kinase A pathway (as the case for β-adrenergic agonists), or protein kinase G (as the case for NO). It is effective in both normal and failing hearts. In patients hospitalized for acute decompensated heart failure, CXL-1020 enhances systolic ejection without altering heart rate and concomitantly lowers left ventricular filling pressures and systemic resistance. This is an attractive constellation of cardiac and systemic hemodynamic effects for acute decompensated heart failure therapy, suggesting that nitroxyl donors may benefit patients with this common and potentially life-threatening disorder.