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A Phase 1 Randomized Study of Single Intravenous Infusions of the Novel Nitroxyl Donor BMS-986231 in Healthy Volunteers

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Abstract

Nitroxyl (HNO) is a reactive nitrogen molecule that has potential therapeutic benefits for patients with acute heart failure. The results of the first-in-human study for BMS-986231, a novel HNO donor, are reported. The aim of this sequential cohort study was to evaluate the safety, tolerability, and pharmacokinetic profile of BMS-986231 after 24- and 48-hour intravenous infusions in healthy volunteers. Eighty subjects were randomized and dosed. Seven cohorts (stratum A) received BMS-986231 0.1, 0.33, 1, 3, 5, 10, and 15 μg/kg/min or placebo, infused over 24 hours. An additional cohort (stratum B) received 10 μg/kg/min or placebo, infused over 48 hours. Adverse events (AEs) were reported for 30 days after completion of infusion. Blood/urine samples were collected at regular intervals; other parameters (blood pressure, heart rate/rhythm, cardiac index) were also assessed. Headaches were the most commonly reported drug-related AE (48%) in those who received BMS-986231, although their severity was reduced by hydration. No other significant drug-related AEs were noted. BMS-986231 was associated with dose-dependent and well-tolerated reductions in systolic and diastolic blood pressure versus baseline; cardiac index, as measured noninvasively, was increased. BMS-986231 had no clinically significant effect on heart rate/rhythm or laboratory parameters. Its mean elimination half-life was 0.7-2.5 hours. BMS-986231 was safe and well-tolerated for up to 24 hours (15 μg/kg/min) or 48 hours (10 μg/kg/min), with a favorable hemodynamic profile observed. Ongoing studies continue to evaluate the potential benefit of BMS-986231 in patients with acute heart failure.

Keywords

heart failure, HNO donor, humans, nitroxyl, pharmacokinetics, phase 1

Each year in the United States alone, >1 million patients are hospitalized with heart failure (HF), with the majority suffering acute decompensated HF.[1–3] The rehospitalization rate among acutely decompensated patients is ~50% after 6 months, and approximately 30% of patients die within 1 year of initial hospitalization.[4]

Current therapies for acute HF (AHF) are significantly limited by hypotension (vasodilators), electrolyte imbalances (diuretics), arrhythmias, and increased mortality (legacy inotropes such as dobutamine and milrinone).[4–7] Thus, there is a need for new therapies that reduce cardiac loads and enhance cardiac output safely and effectively.

Nitroxyl (HNO) mediates reversible biological effects by interacting directly with specific thiolates on target proteins. In the cardiovascular system, it induces positive inotropy and improved lusitropy via cyclic AMP-independent modifications of sarco-/endoplasmic reticulum Ca^{2+}-ATPase, phospholamban, and the ryanodine receptor, resulting in increased calcium cycling without elevation of intracellular calcium[8–11] or increased L-type calcium channel activity.[12] HNO also modifies cardiac myofilaments to increase calcium sensitivity. In the periphery, HNO regulates soluble guanylate cyclase to effect vasodilation.[13] Thus, the net effect is improved cardiac function via direct augmentation of inotropy/lusitropy and increased peripheral vasodilation, suggesting HNO's utility in AHF.

In patients with advanced HF, 6-hour infusions of a first-generation HNO donor, CXL-1020, elicited dose-dependent decreases in pulmonary capillary wedge pressure (PCWP) and increases in the cardiac index,

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with no meaningful change in heart rate[14]; however, CXL-1020 was associated with infusion-site toxicity, and development was halted. Adverse events (AEs) such as arrhythmias have not been associated with HNO donors.[14–17]

BMS-986231 (formerly CXL-1427), a novel second-generation HNO donor, delivers HNO via pH-dependent chemical breakdown when exposed to the neutral pH environment of the bloodstream. Increasing BMS-986231 dose infusions in dogs with induced cardiomyopathy resulted in reductions in mean arterial pressure, systemic vascular resistance, Tau, and the end-diastolic pressure volume relationship; increased left ventricular ejection fraction/fractional area shortening, end-systolic pressure volume, and preload-recruitable stroke work relationship parameters were also noted.[18] These findings suggest that BMS-986231 possesses positive lusitropic and inotropic as well as vasodilatory effects. There was no increase in heart rate or myocardial oxygen consumption, and no de novo arrhythmic activity was detected.[18]

The primary objective of this phase 1 first-in-human study was to evaluate the safety and tolerability of 24- and 48-hour intravenous infusions of BMS-986231 in healthy volunteers. The secondary objective was to establish the pharmacokinetic (PK) profile of BMS-986231. Given the expected pharmacodynamic effect of HNO, the study explored changes in non-invasively measured hemodynamic parameters with BMS-986231.

Methods

Study Design and Population

This study was conducted in compliance with good clinical practice as described in the Food and Drug Administration's Code of Federal Regulations and the International Conference on Harmonisation's "Guidance for Industry" document, and in accordance with the Declaration of Helsinki. Written approval for the protocol and informed consent form were obtained from the Duke Health Institutional Review Board. Written informed consent was obtained from all participants.

This was a randomized, double-blind, placebo-controlled study. Cohorts of 10 healthy volunteers each (18-45 years; body mass index, 18-34 kg/m^2; weight, 50-110 kg) were sequentially enrolled for ascending doses of BMS-986231 (Supplementary Figure S1). A full list of inclusion and exclusion criteria is included in the Supplementary Appendix. In stratum A, 7 dose-infusion cohorts (A1 to A7, BMS-986231 0.1, 0.33, 1, 3, 5, 10, and 15 μg/kg/min, respectively) were assessed over 24 hours of continuous infusion. In stratum B, 1 cohort (10 μg/kg/min) was assessed over 48 hours of continuous infusion (cohort B1). Preclinical studies were used to determine the initial 0.1-μg/kg/min dose; subsequent doses were determined by the principal investigator and medical monitor based on cumulative safety data in preceding cohorts. The maximum tolerated dose over 24 hours in stratum A was assessed over 48 hours in stratum B.

Ten subjects were randomized in each cohort (n = 6 [BMS-986231] to n = 4 [placebo]). The randomization procedure was an integrated function of the electronic data capture system. After subjects were randomized, treatment assignments were made available to the unblinded investigational pharmacist(s) responsible for the preparations of the blinded dosing solutions at the study site. Study subjects, the principal investigator, and all other clinical staff members at the Duke Early Phase Clinical Research Unit (Durham, North Carolina) were blinded to study drug assignments until after study completion.

Solutions for intravenous infusion contained BMS-986231 with Captisol (sulfobutylether-beta cyclodextrin; Ligand, Inc., San Diego, California) to enhance solubility; 5% dextrose injection (United States Pharmacopeia [USP]); and potassium acetate injection (USP). Dosing solutions of <1 mg/mL were prepared without potassium acetate for improved stability. Placebo did not contain Captisol but was visually indistinguishable from active drug solution.

All procedures/assessments were carried out at the Duke Early Phase Clinical Research Unit. After 24 hours (stratum A) or 48 hours (stratum B) of continuous infusion, subjects were observed for an additional 24 hours prior to discharge. Return visits were scheduled for 2 days after discharge and on day 15 from study start (Supplementary Table S1). Thirty days after termination of infusion, all subjects were contacted by telephone to assess for additional or unresolved AEs.

Safety Assessments and Pharmacokinetic/Pharmacodynamic Evaluations

Adverse Events. All AEs from informed consent through 30 days after completion of infusion were documented.

Laboratory Tests. Blood and urine samples were collected at specified intervals (Supplementary Table S1). BMS-986231 and BMT-284730 concentrations (the principal and inactive metabolite of BMS-986231) were measured (XenoBiotic Laboratories, Inc., Plainsboro, New Jersey) using validated liquid chromatography with tandem mass spectrometry methods. A protein precipitation procedure was used to extract the analytes from 50 μL of plasma sample (acidified with citric acid) or 250 μL of urine sample. The extract was then subjected to reverse-phase high-performance

liquid chromatography, and detection of the analytes was performed by tandem mass spectrometry. The assay calibration curves ranged from 5.0 to 2500 ng/mL for BMS-986231 and BMT-284730 in plasma samples, and from 25.0 to 12 500 ng/mL for BMS-986231 and BMT-284730 in urine samples.

Routine hematology, serum chemistry, coagulation, and urinalysis parameters were also assessed (Supplementary Table S1 and supplementary appendix). To evaluate the potential effects of BMS-986231 on creatinine clearance and urinary excretion of electrolytes and aldosterone, additional assessments were performed in stratum A cohorts only.

Twelve-Lead Electrocardiography. Continuous 12-lead electrocardiogram (ECG) with central station monitoring was performed. Triplicate static 12-lead ECG reports/tracings were also generated at prespecified times (Supplementary Table S1).

Physical Examination, Blood Pressure, Heart Rate, Body Temperature, Cardiac Index. Physical examinations were undertaken at specified intervals (Supplementary Table S1). Blood pressure and heart rate were also measured regularly during the infusions and at subsequent study visits (Supplementary Table S1).

Cardiac index was measured on an exploratory basis in stratum A only (Supplementary Table S1), using a noninvasive cardiac output monitor (NICaS, NI Medical USA, Chapel Hill, North Carolina).

Statistical Analyses

The sample size for this study was selected empirically. The number of subjects in each cohort (6 subjects randomized to receive BMS-986231 and 4 subjects randomized to receive placebo) was deemed to be sufficient to allow for an initial assessment of the safety and PK properties of 24- and 48-hour infusions of BMS-986231 at various doses and to minimize exposure to the drug in healthy human subjects. The safety population was defined as all randomized subjects receiving an infusion. The PK population consisted of all safety population subjects with evaluable plasma samples.

Categorical data are expressed as counts and percentages and continuous data with descriptive statistics. Results for individual BMS-986231 dosage groups within each stratum are reported; placebo results are combined within each stratum. All analyses were performed using STATA version 13.0 (College Station, Texas). Coding of AEs and medications was performed using MedDRA v17.0 and WHO-DDE Mar2014 dictionaries. Plasma PK parameters, including steady-state plasma concentration (C_{ss}), area under the plasma concentration-versus-time curve ($AUC_{0-\infty}$), apparent terminal half-life ($t_{1/2}$), total clearance (CL), renal clearance, and volume of distribution (V_z) were derived (Supplementary Table S2). Standard noncompartmental analysis methods were used (WinNonlin v6.3, Pharsight Corporation, Sunnyvale, California) and results summarized with descriptive statistics.

Results

Subject Disposition

Of the 83 subjects randomized, 80 were dosed (BMS-986231, n = 48; placebo, n = 32; Figure 1). The mean durations of BMS-986231 and placebo exposures were 23.9 and 24.1 hours (stratum A) and 48.1 and 48.3 hours (stratum B), respectively. Subject demographics at baseline were similar across treatment cohorts and in those receiving active treatment and placebo (Table 1).

In stratum A, 3 subjects from cohort A4 (all on 3 μg/kg/min of active drug) discontinued treatment ~21-22 hours into the infusion period because of headaches. No other AEs were associated with treatment discontinuation. In the same cohort, 1 subject randomized to placebo experienced infusion catheter displacement at approximately 21 hours; the catheter was reinserted, but the amount of infusion time/volume lost was unknown. All 4 subjects completed the subsequent follow-up period. An additional 2 subjects (1 placebo, 1 stratum B) withdrew consent postinfusion and did not complete study follow-up.

Safety and Tolerability

Treatment-Emergent Adverse Events. Approximately 80% of subjects experienced a treatment-emergent AE (TEAE). No serious or fatal TEAEs were observed. In stratum A, the overall TEAE incidence ranged from 3 of 6 subjects to 6 of 6 subjects across BMS-986231 groups and 22 of 28 receiving placebo. In stratum B, 6 of 6 subjects receiving BMS-986231 and 3 of 4 receiving placebo experienced a TEAE.

TEAE patterns were comparable between subjects. The most common TEAE was contact dermatitis (erythema, pruritus, or hyperpigmentation) from the adhesive pads used for monitoring cardiac function or the tape used to secure intravenous catheters (Table 2); these were not considered drug related.

Headaches were reported in 23 of 48 subjects receiving BMS-986231 (48%) and 5 of 32 receiving placebo (16%); see Table 2. These were considered vascular in nature by the investigator and were sometimes associated with nausea or lightheadedness. All 23 cases were considered related or possibly/probably related to the study drug. Three cases of headache in subjects given placebo were considered related or possibly related to treatment. Headaches reported in the 3-μg/kg/min cohort (A4) led to discontinuation of infusion in 3 of these subjects. In subsequent higher-dose cohorts, oral hydration ad libitum was encouraged after it was

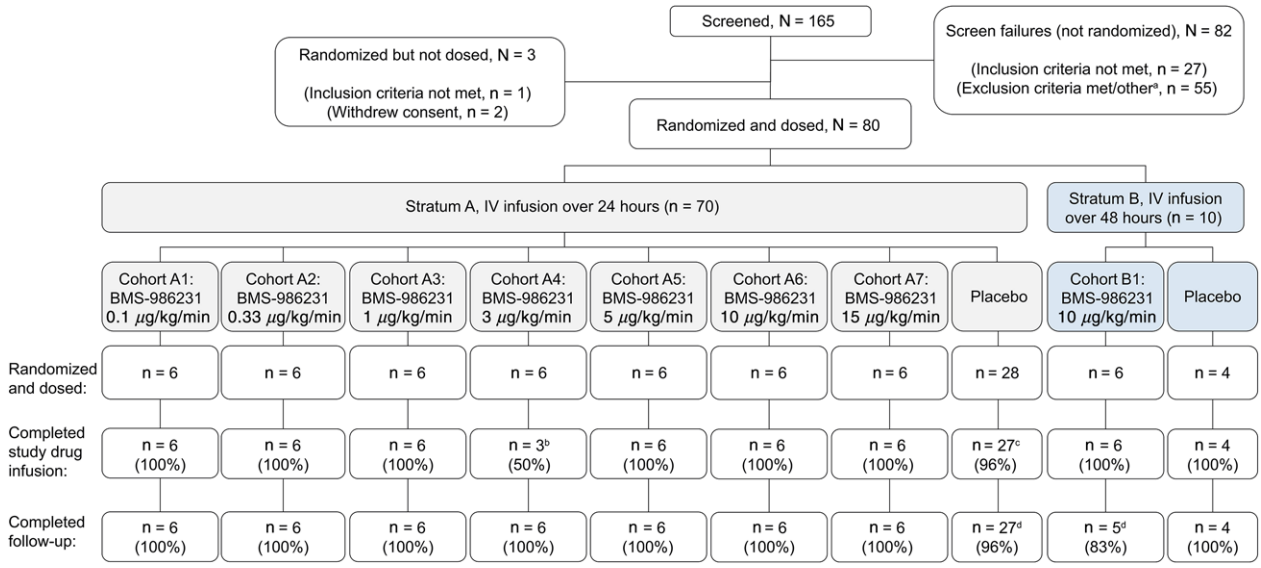

Figure 1. Subject flow. [a]Any other reason in the investigator's opinion that would make it inappropriate for the subject to participate. [b]Three subjects did not complete the infusion because of headache. [c]One subject did not receive a full 24 hours of intravenous placebo infusion because of catheter displacement at approximately hour 21 (lost infusion time/volume unknown — see text). [d]One subject withdrew consent during follow-up. IV, intravenous.

reported by investigators to reduce headache severity; intravenous hydration was also administered as needed for headache relief.

Seven subjects receiving BMS-986231 (15%) experienced transient and self-limited infusion-site erythema compared with 3 receiving placebo (9%); see Table 2. These were predominantly painless. One of these (cohort A6 [10 μg/kg/min]) was associated with mechanical trauma. There was no indication of dose or exposure dependence for the rate of erythema occurrence. Late-onset thrombophlebitis was seen in 2 subjects. Subject 1 (BMS-986231 1 μg/kg/min) experienced phlebitis in the contralateral arm (site of the peripheral intravenous tube placed for blood draws), beginning on day 9 and developing into thrombophlebitis with a palpable cord on day 50. The investigator did not consider this to be study drug related because the BMS-986231 infusion site was unaffected. Subject 2 (15 μg/kg/min) developed painless infusion-site erythema, which was resolving on the day 5 visit. This subject then experienced thrombophlebitis on day 12 in the ipsilateral arm, which was considered possibly related to the study drug.

Severe TEAEs were reported in 4 of 48 subjects (8%) given BMS-986231; 3 of these were reported as "headache" (3, 15, and 10 μg/kg/min [cohort B1]) and 1 as "vascular headache" (10 μg/kg/min [cohort A6]). One stratum A subject receiving placebo (4%) experienced an increase in blood creatine phosphokinase that was considered severe; this occurred 2 weeks after the placebo infusion and was recorded as being "secondary to heavy exercise."

Clinical Laboratory Evaluation. No clinically significant changes in hemoglobin or hematocrit were seen overall. There were no other clinically significant dose- or exposure-dependent changes in hematologic, coagulation, or metabolic laboratory parameters (Supplementary Table S3).

Mean C-reactive protein (CRP) appeared to increase above baseline across all stratum A cohorts (including placebo) postinfusion. The increase appeared to be greater in subjects receiving study drug compared with placebo. CRP increases were transient and without clinical sequelae, with levels typically returning to baseline during follow-up. In stratum B, similar transient changes in mean CRP without clinical sequelae were observed.

Twelve-Lead ECG Findings. There were no clinically significant dose- or exposure-dependent changes in Fridericia-corrected QT (QTcF) in cohorts treated with BMS-986231 (Supplementary Table S4). Mean QTcF values for BMS-986231 and placebo groups did not clinically differ. No drug- or dose-related trends were seen for other ECG intervals (PR, QRS).

Pharmacodynamic Evaluations. Mean baseline systolic blood pressures (SBPs) were similar across cohorts A1-A7 (122 mm Hg [BMS-986231 combined], 118 mm Hg [placebo]; Supplementary Figure S2A). A dose-dependent reduction in SBP was seen with BMS-986231, beginning 2 hours after infusion start (Figure 2A). With the 15-μg/kg/min dose, mean and maximum reductions in SBP at 24 hours were 11.5

Table 1. Subject Disposition at Baseline

	Stratum A, Continuous Intravenous Infusion Over 24 Hours								Stratum B, Continuous Intravenous Infusion Over 48 Hours			
	BMS-986231, Cohort (μg/kg/min)											
	A1 (0.1)	A2 (0.33)	A3 (1)	A4 (3)	A5 (5)	A6 (10)	A7 (15)	Placebo	BMS-986231, Cohort B1 (10 μg/kg/min)	Placebo	BMS-986231 (Strata A and B Combined)	Placebo (Strata A and B Combined)
Subjects, n	6	6	6	6	6	6	6	28	6	4	48	32
Sex, n (%)												
Women	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (14.3)	0 (0)	0 (0)	1 (2.1)	4 (12.5)
Men	5 (83.3)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	24 (85.7)	6(100)	4 (100)	47 (97.9)	28 (87.5)
Age, mean years (± SD)	31.6 (7.4)	30.8 (7.4)	33.3 (8.8)	33.7 (10.0)	27.5 (3.2)	31.0 (6.8)	36.2 (8.0)	33.5 (7.0)	26.6 (5.5)	30.1 (3.4)	31.3 (7.5)	33.1 (6.7)
Race, n (%)												
White	1 (16.7)	3 (50)	3 (50)	1 (16.7)	2 (33.3)	4 (66.7)	2 (33.3)	11 (39.3)	2 (33.3)	2 (50)	18 (37.5)	13 (40.6)
Black or African American	5 (83.3)	2 (33.3)	3 (50)	5 (83.3)	4 (66.7)	2 (33.3)	4 (66.7)	16 (57.1)	4 (66.7)	2 (50)	29 (60.4)	18 (56.3)
Mixed race		1 (16.7)						1 (3.6)			1 (2.1)	1 (3.1)
Weight, mean kg (± SD)	83.4 (12.0)	87.5 (5.4)	82.3 (7.4)	84.6 (9.5)	79.4 (8.7)	76.1 (10.3)	86.0 (9.9)	82.6 (10.0)	85.1 (12.9)	82.2 (8.4)	83.1 (9.7)	82.5 (9.7)
Height, mean cm (± SD)	171.4 (9.3)	181.0 (6.6)	174.8 (10.3)	178.3 (2.6)	173.9 (7.4)	178.6 (3.4)	172.5 (9.8)	178.0 (8.0)	175.4 (6.0)	178.0 (3.6)	175.7 (7.5)	178.0 (7.5)
BMI, mean kg/m^2 (± SD)	28.3 (2.5)	26.8 (2.9)	27.1 (3.7)	26.6 (2.8)	26.4 (3.4)	23.8 (2.6)	29.0 (3.1)	26.1 (3.1)	27.8 (4.9)	25.9 (1.8)	27.0 (3.4)	26.1 (2.9)

BMI, body mass index; SD, standard deviation.

Table 2. Treatment-Emergent Adverse Events (TEAEs) Reported for ≥2 Subjects (All Cohorts, Independent of Treatment Assignment)

	Stratum A, Continuous Intravenous Infusion Over 24 Hours								Stratum B, Continuous Intravenous Infusion Over 48 Hours			
	BMS-986231, Cohort (μg/kg/min)											
TEAE	A1 (0.1)	A2 (0.33)	A3 (1)	A4 (3)	A5 (5)	A6 (10)	A7 (15)	Placebo	BMS-986231, Cohort B1 (10 μg/kg/min)	Placebo	BMS-986231 (Strata A and B Combined)	Placebo (Strata A and B Combined)
Subjects, n	6	6	6	6	6	6	6	28	6	4	48	32
Gastrointestinal disorders												
Diarrhea, n (%)	0	0	0	1 (16.7)	0	0	0	1 (3.6)	0	0	1 (2.1)	1 (3.1)
Nausea, n (%)	0	0	0	2 (33.3)	1 (16.7)	4 (66.7)	1 (16.7)	3 (10.7)	0	0	8 (16.7)	3 (9.4)
Vomiting, n (%)	0	0	0	1 (16.7)	0	1 (16.7)	0	1 (3.6)	0	0	2 (4.2)	1 (3.1)
General disorders and administration-site conditions												
Infusion-site erythema, n (%)	0	0	0	0	0	3 (50)[a]	2 (33.3)	1 (3.6)	2 (33.3)	2 (50)	7 (14.6)[a]	3 (9.4)
Vessel puncture site erythema, n (%)	0	0	0	0	0	0	2 (33.3)	0	0	0	2 (4.2)	0
Injury, poisoning, and procedural complications												
Skin injury, n (%)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)	3 (10.7)	1 (16.7)	1 (25)	9 (18.8)	4 (12.5)
Nervous system disorders												
Dizziness, n (%)	1 (16.7)	0	0	0	0	1 (16.7)	0	1 (3.6)	1 (16.7)	0	3 (6.3)	1 (3.1)
Postural dizziness, n (%)	0	0	0	0	1 (16.7)	2 (33.3)	0	0	0	0	3 (6.3)	0
Headache, n (%)	0	1 (16.7)	2 (33.3)	4 (66.7)	3 (50)	3 (50)	5 (83.3)	5 (17.9)	5 (83.3)	0	23 (47.9)	5 (15.6)
Respiratory, thoracic, and mediastinal disorders												
Nasal congestion, n (%)	0	1 (16.7)	0	0	0	0	1 (16.7)	0	0	0	2 (4.2)	0
Skin and subcutaneous tissue disorders												
Contact dermatitis, n (%)	1 (16.7)	2 (33.3)	4 (66.7)	4 (66.7)	4 (66.7)	2 (33.3)	4 (66.7)	15 (53.6)	3 (50)	2 (50)	24 (50)	17 (53.1)

[a]Associated with mechanical trauma in 1 subject.

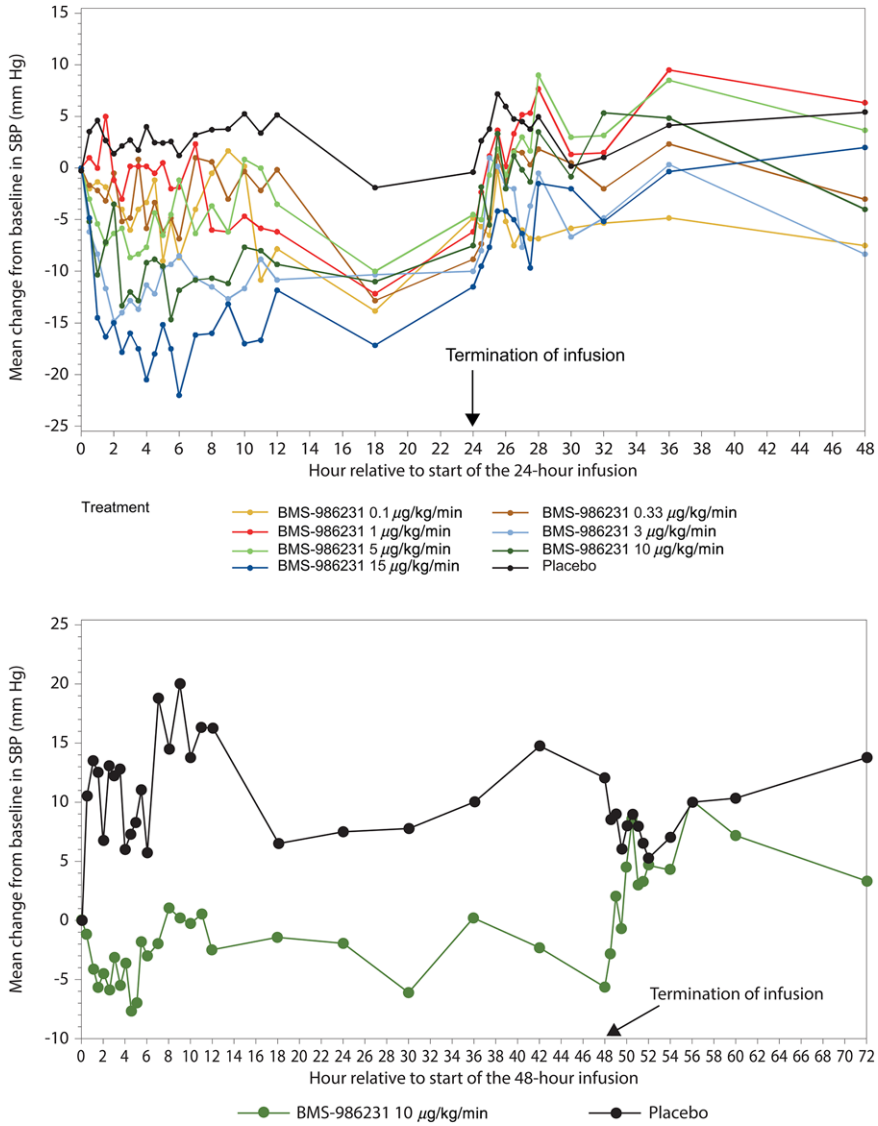

Figure 2. Mean changes from baseline in systolic blood pressures over time. (A) Stratum A, 24-hour infusion. (B) Stratum B, 48-hour infusion. SBP ranges at baseline (active treatment): 103-153 mm Hg (stratum A) and 112-126 mm Hg (stratum B). Mean SBPs over time are shown in Supplementary Figure S2. SBP, systolic blood pressure.

and 43 mm Hg, respectively. SBP levels began to recover within 0.5 hours of study drug cessation and approached baseline levels within 1.5 hours of cessation. Subjects given placebo experienced little change in SBP during and after infusion. Stratum B subjects administered BMS-986231 10 μg/kg/min experienced similar and persistent SBP reductions throughout the 48 hours of infusion (Figure 2B and Supplementary Figure S2B); mean and maximum reductions in SBP at 48 hours were 5.7 and 16 mm Hg, respectively. After discontinuation, the time course of SBP recovery was similar to that seen over 24 hours in the stratum A 10-μg/kg/min cohort.

Dose-dependent reduction in diastolic blood pressure (DBP) was also observed with BMS-986231 (Supplementary Figure S3A,B). Mean baseline DBP across all groups was 71 mm Hg (Supplementary Figure S4A,B). Mean and maximum DBP reductions with BMS-986231 15 μg/kg/min at 24 hours were 14.8 and 33 mm Hg, respectively. Corresponding mean and maximum DBP reductions in stratum B at 48 hours were 6.8 and 14 mm Hg, respectively. Minimal changes were observed with placebo. DBP recovered toward baseline after BMS-986231 infusion. Reductions in blood pressure were clinically well tolerated.

In stratum A, mean baseline heart rates averaged 62 and 59 beats per minute (bpm) for BMS-986231 and placebo, respectively (Supplementary Figure S5A). In stratum B, these were 51 and 55 bpm, respectively (Supplementary Figure S5B). Although there were no

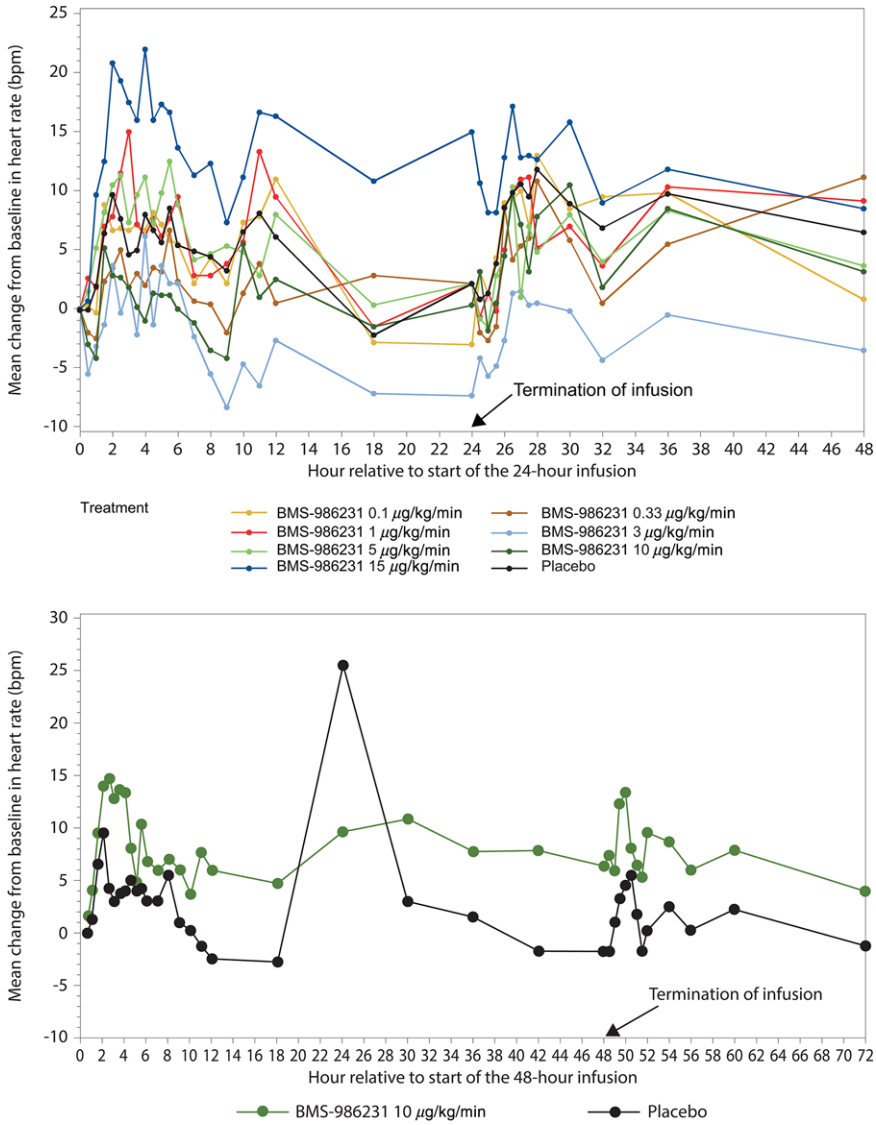

Figure 3. Mean changes from baseline in heart rates over time. (A) Stratum A, 24-hour infusion. (B) Stratum B, 48-hour infusion. HR ranges at baseline (active treatment): 43-98 bpm (stratum A) and 40-59 bpm (stratum B). Mean HRs over time are shown in Figure S5. bpm, beats per minute; HR, heart rate.

dose-dependent increases in heart rate, increases from baseline were more apparent in the 15-μg/kg/min cohort. Three subjects (placebo and 5- and 15-μg/kg/min cohorts) had heart rates >100 bpm during infusion, although they returned to baseline within 24 hours after the infusion or during follow-up. Overall, there was no evidence of a dose- or exposure-dependent response in heart rate (Figure 3A). In stratum B, there were no notable differences in the change in mean heart rate during the infusion and subsequent 24 hours between BMS-986231 and placebo (Figure 3B). Mean heart rates were within normal range for all dose cohorts.

The mean baseline noninvasive cardiac index (cohorts A1-A7 only) was 3.45 and 3.37 L/min/m^2 for BMS-986231 and placebo, respectively. There was a dose-dependent rise in cardiac index during infusion, with a mean 38% increase observed with BMS-986231 15 μg/kg/min after 2 hours (Figure 4). Placebo cardiac index remained relatively constant throughout the infusion. Six hours postinfusion, cardiac index remained 18% and 26% above baseline with BMS-986231 10 and 15 μg/kg/min, respectively.

Pharmacokinetic Assessments

Mean BMS-986231 plasma concentration-time profiles are presented in Figure 5A,B. Summary statistics of BMS-986231 PK parameters by cohort are presented in Table 3. Mean BMT-284730 plasma concentration-time profiles are shown in Figure 6A,B. Summary statistics of BMT-284730 PK parameters by cohort are presented in Supplementary Table S5.

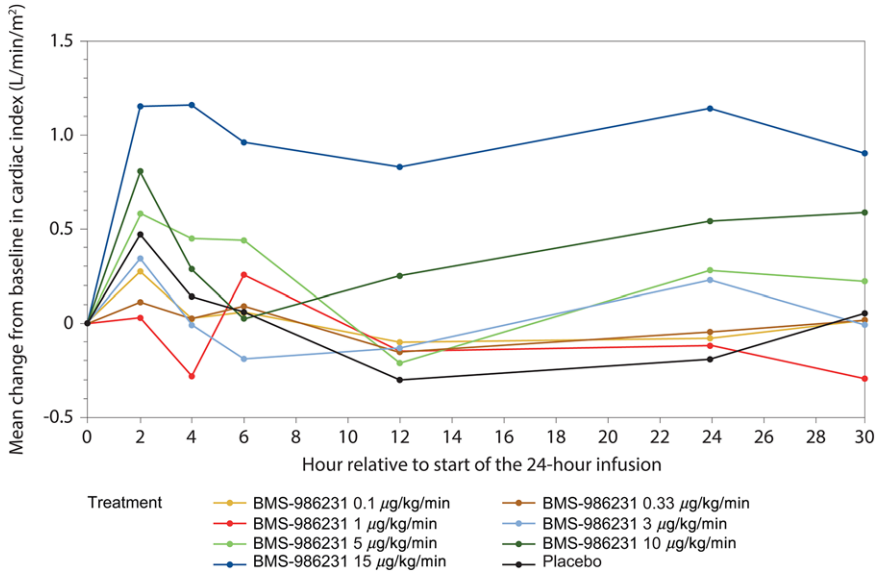

Figure 4. Mean change from baseline in cardiac index over time (stratum A only).

Mean plasma BMS-986231 and BMT-284730 concentrations in stratum A increased with infusion rate from 0.1-15 μg/kg/min (Figures 5A,B and 6A,B).

The mean $t_{1/2}$ of BMS-986231 was 0.7-2.5 hours, decreasing substantially with increasing dose (Table 3). A greater-than-proportional increase in $AUC_{0-\infty}$ for BMS-986231 was seen with increasing dose; increases in C_{ss} were also greater than proportional relative to the increase in BMS-986231 dose. There was a mean decrease of approximately 60% in CL with increasing BMS-986231 dose from 0.33 μg/kg/min (geometric mean, 191 L/h) to 15 μg/kg/min (79 L/h) and a decrease of approximately 90% in V_z over the same dose range (Table 3). A graph showing geometric mean $AUC_{0-\infty}$ values for BMS-986231 relative to dose infusion rate is included in the supplementary appendix (Supplementary Figure S6).

Analysis of the pooled urine samples showed that 0.39% to 0.78% of the administered BMS-986231 dose was present as unchanged drug. BMT-284730 was calculated to represent 17.1 to 26.1% of the administered BMS-986231 dose. The ratio of $AUC_{0-\infty}$ values for BMT-284730 relative to BMS-986231 ranged from 10.4 (cohort A2) to 3.91 (cohort A7).

Discussion

BMS-986231 is a novel HNO donor that induces positive inotropy and lusitropy, along with vasodilatory effects, through mechanisms distinct from any currently available HF therapies.[8,10,12–19] Animal HF models have demonstrated that BMS-986231 reduces peripheral resistance and enhances cardiac contractility without increasing myocardial oxygen consumption, heart rate, or risk of arrhythmias.[18]

In this first-in-human study, BMS-986231 was well tolerated for up to 24 hours (continuous 15 μg/kg/min infusion) and 48 hours (10 μg/kg/min infusion). Headache was the most common drug-related TEAE. Headache is a common side effect of vasodilators[4,6]; this was not an unexpected finding. Most headaches were considered mild to moderate and appeared to be mitigated by supplemental hydration. Notably, the 3 subjects who discontinued because of headache (cohort A4) were considered clinically hypovolemic at the time. Subsequent cohorts that received supplemental oral or intravenous hydration (to prevent headaches or reduce their intensity) experienced considerably fewer headaches with no discontinuations of study drug. In a subsequent phase 2a study, mild to moderate headaches were also reported in patients hospitalized with decompensated HF and reduced ejection fraction receiving 6-hour BMS-986231 infusions (3-12 μg/kg/min); these did not lead to interruption or early termination of infusion.[20]

BMS-986231 was associated with dose-dependent blood pressure reductions at doses ≥3 μg/kg/min, consistent with both its activity as a vasodilator[18] and with earlier CXL-1020 studies.[14] These reductions were rapid and sustained, and began returning to baseline shortly after infusion termination. There was only 1 case of asymptomatic hypotension with BMS-986231 (3 μg/kg/min), which was moderate, considered "probably related" to study drug, and resolved without intravenous hydration. Blood pressure reductions were well tolerated. Similarly, in the later phase 2a study there was 1 case of asymptomatic hypotension (moderate) that was considered drug related and no cases of symptomatic hypotension.[20]

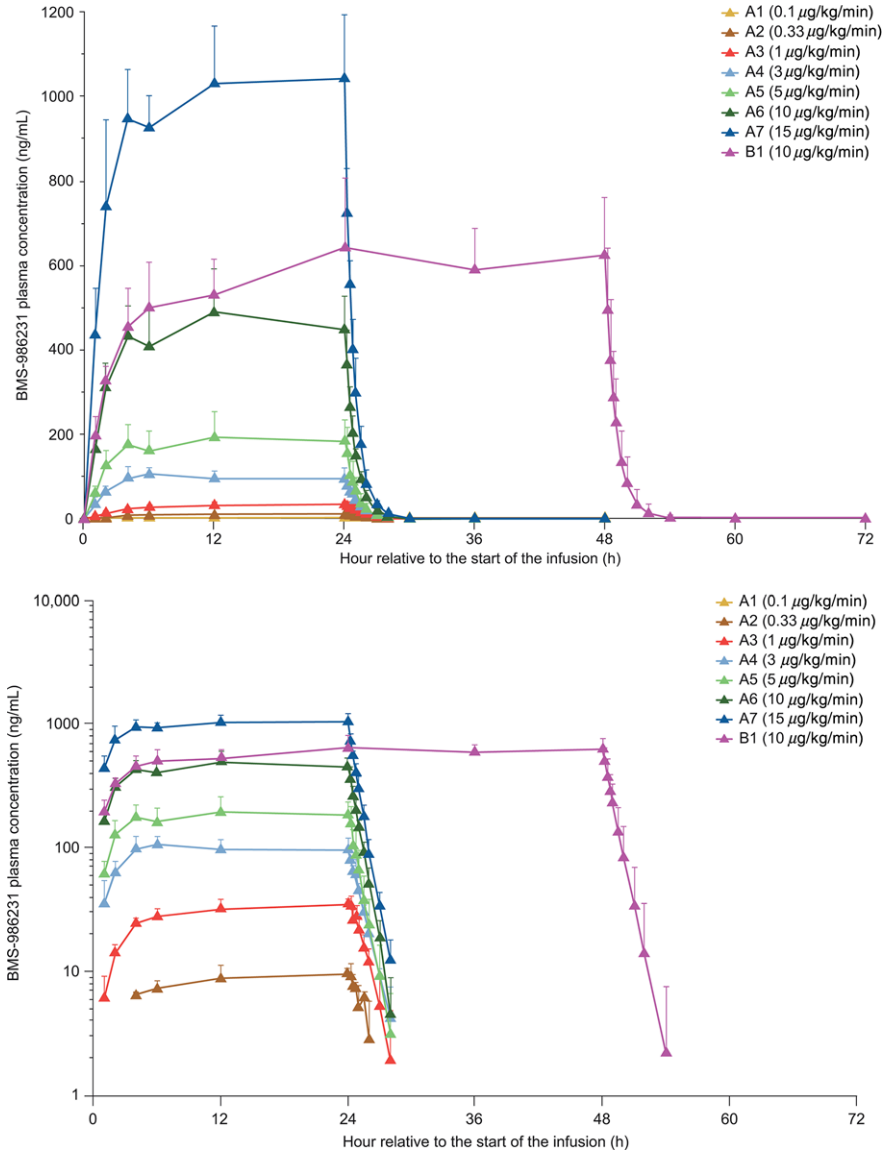

Figure 5. Mean ± SD BMS-986231 plasma concentration-time profiles after intravenous administration. (A) Linear plot. (B) Log-linear plot. SD, standard deviation.

There was no clinically significant effect of BMS-986231 on ECG parameters or heart rate. Cardiac index assessment suggested that cardiac output increased, particularly with the 15-μg/kg/min dose. The NICaS method has been validated elsewhere,[21] and increased cardiac index without an effect on heart rate is consistent with BMS-986231 inotropy in animal models.[18] This was also reflected in the phase 2a study, in which BMS-986231 was associated with significant increases in NICaS-assessed stroke volume index and cardiac index versus placebo at the higher doses.[20]

There was no clear indication of venotoxicity. The incidence of overt thrombophlebitis was consistent with the presence and duration of indwelling catheters, such as those used for repeat phlebotomy and infusion of study drug/placebo.[22] There was also no clinically significant effect of BMS-986231 on most laboratory parameters. Consistent with its properties as a highly sensitive acute-phase reactant, changes in CRP level were increased and were greater in subjects receiving study drug compared with placebo; however, contact dermatitis resulting from the ECG/monitor adhesive strips was noted frequently and may have been a confounding factor. Increased CRP levels were also transient and without clinical sequelae.

Plasma exposure (C_{ss} and AUC) of BMS-986231 increased with infusion rate and dose in the dose range of 0.1-15 μg/kg/min. Estimates of exposure parameters revealed an apparent trend toward a greater-than-proportional increase in exposure at doses above

Table 3. BMS-986231 Parameters by Cohort

Parameter	Cohort	Dose Rate (μg/kg/min)	Geometric Mean
$AUC_{0-\infty}$ (μg·h/mL)	A2	0.33	0.219
	A3	1	0.748
	A4	3	2.20
	A5	5	4.12
	A6	10	10.6
	A7	15	23.4
	B1	10	27.5
C_{ss} (μg/mL)	A2	0.33	0.009
	A3	1	0.031
	A4	3	0.092
	A5	5	0.172
	A6	10	0.441
	A7	15	0.976
	B1	10	0.572
V_z (L)	A2	0.33	677
	A3	1	286
	A4	3	220
	A5	5	138
	A6	10	97.9
	A7	15	74.7
	B1	10	87.1
CL (L/h)	A2	0.33	191
	A3	1	159
	A4	3	167
	A5	5	138
	A6	10	102
	A7	15	79.0
	B1	10	88.8
CL_r (L/h)	A2	0.33	0.69
	A3	1	0.80
	A4	3	0.74
	A5	5	0.53
	A6	10	0.64
	A7	15	0.60
	B1	10	0.56
$t_{1/2}$ (h)	A2	0.33	2.5
	A3	1	1.3
	A4	3	0.9
	A5	5	0.7
	A6	10	0.7
	A7	15	0.7
	B1	10	0.7

$AUC_{0-\infty}$, area under the concentration-time curve from time zero extrapolated to infinity; CL, total body clearance; CL_r, renal clearance; C_{ss}, steady-state plasma concentration; $t_{1/2}$, apparent terminal half-life; V_z, volume of distribution.

5 μg/kg/min (see Supplementary Figure S6). Preclinical in vitro blood cell partitioning experiments have shown that BMS-986231 partitions highly into red blood cells (RBCs) in rat, dog, and human blood, with blood-to-plasma (CB/CP) ratios much higher than 1 (data not shown). In these experiments, the uptake of BMS-986231 was concentration dependent, with the CB/CP ratio for humans approximately 10-fold at plasma concentrations up to 0.5 μM and decreasing to 5- and 1.5-fold at plasma concentrations of 10 and 65 μM, respectively. For reference, plasma steady-state levels of BMS-986231 in the human dose-escalation study were approximately 1, 2.5, and 5.5 μM at the 5-, 10-, and 15-μg/kg/min dose, respectively. Therefore, at doses of 10 and 15 μg/kg/min, the CB/CP ratios are approximately 5-fold, representing a 2-fold decrease in the CB/CP ratios compared with the 5-μg/kg/min dose. Greater levels of BMS-986231 in plasma, because of a 2-fold decrease in blood-to-plasma ratio, may therefore explain the departure from proportionality at doses above 5 μg/kg/min.

The urinary recovery of BMS-986231 was $<1\%$ of the dose in each cohort, indicating that it is principally eliminated by chemical conversion to inactive BMT-284730 or through metabolism. Conversion of BMS-986231 to HNO and its inactive byproduct have been identified as important elimination routes. Although conversion to BMT-284730 is nonenzymatic, BMS-986231 is metabolized via reduction of the N-hydroxyl group (forming the sulfonamide BMT-279554) and via direct glucuronidation. In vitro data suggest that mitochondrial amidoxime reductase components 1 and 2 are likely to play a role in the formation of BMT-279554 (data not shown). Mitochondrial amidoxime reductase components 1 and 2 are ubiquitous in humans and unlikely to be saturated at the micromolar concentrations of BMS-986231 in plasma. Thus, concentration-dependent differences in blood-to-plasma ratio at high doses, as opposed to saturation of the clearance pathway, is believed to be the reason for the observed departure from dose-proportionality of BMS-986231.

The pharmacokinetics of BMS-986231 were characterized by rapid elimination and a short half-life (0.7 to 2.5 hours) in plasma in the dose range of 0.1 to 15 μg/kg/min.

Paradoxically, there was a consistent trend of shorter half-life as the rate of infusion increased (Table 3). A longer half-life is generally expected for a drug that shows lower clearance at higher doses, but the half-life of BMS-986231 was shorter, with lower CL at higher rates of infusion. This was because the decrease in V_z was greater than the decrease in CL at the various doses studied (Table 3). Previous nonclinical studies suggested the underlying cause of these changes in CL, half-life, and V_z with infusion rate of BMS-986231 was the binding of BMS-986231 to carbonic anhydrase and its partitioning into RBCs, where it is stabilized (data not shown). At low infusion rates, a higher proportion of the BMS-986231 would reside in RBCs, resulting in a high V_z and longer apparent half-life. At higher infusion rates, the binding of BMS-986231 to carbonic anhydrase would begin to saturate, V_z would drop significantly, and a greater proportion of the BMS-986231 dose would be free in the plasma and not stabilized, resulting in a shorter apparent

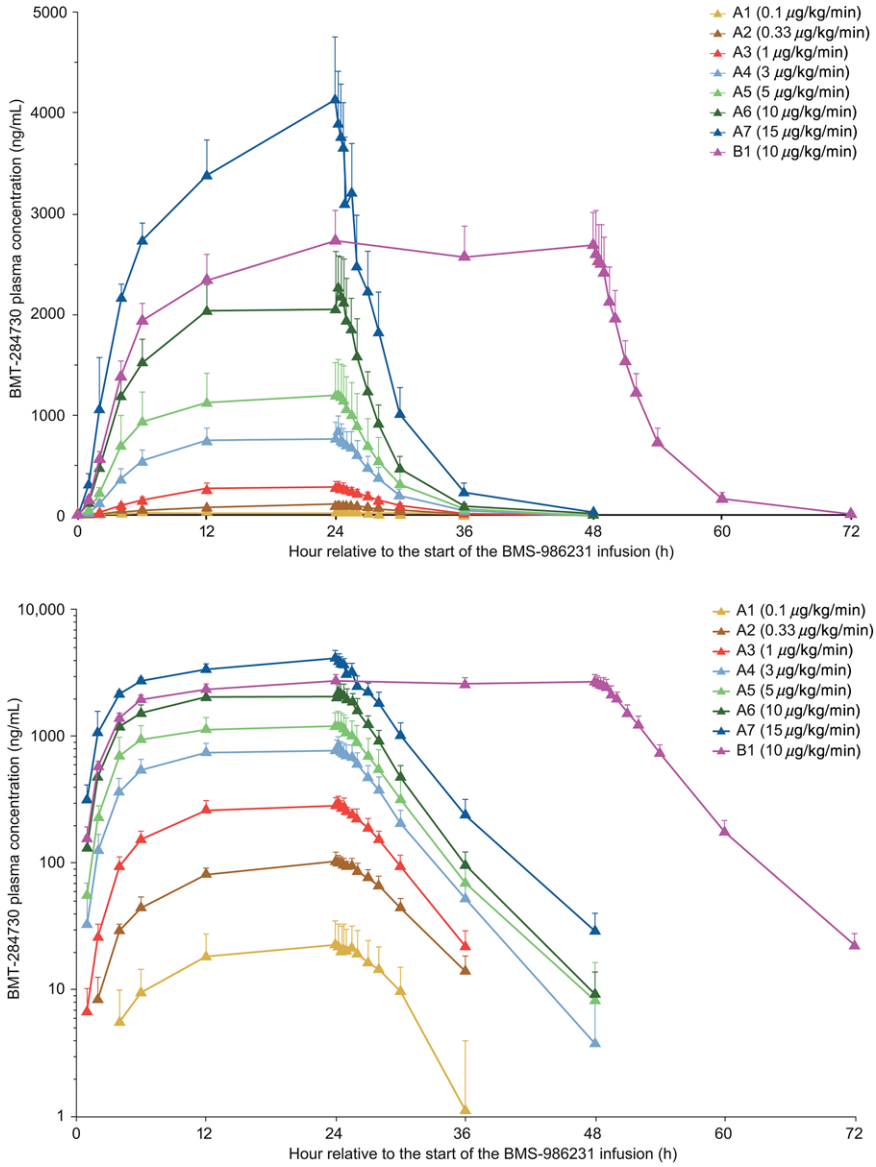

Figure 6. Mean ± SD BMT-284730 plasma concentration-time profiles after intravenous administration of BMS-986231. (A) Linear plot. (B) Log-linear plot. SD, standard deviation.

half-life. This saturation of binding to carbonic anhydrase and consequent decrease in RBC partitioning is also consistent with the dose disproportionality of C_{ss} and $AUC_{0\text{-}\infty}$.

There are some limitations associated with the study. Oral hydration for subjects receiving the higher BMS-986231 doses may have impacted the blood pressure observed. Intravenous hydration was used only as needed, but when given, generally entailed administration of 500 mL of saline over 1 hour, with some subjects in the highest dose group receiving up to 2 L. Hydration was not administered in response to changes in blood pressure, and although the 15-μg/kg/min dose was well tolerated, it was reasoned that fluid supplementation may have impacted blood pressure. As a result, 15 μg/kg/min for 24 hours was inferred to be a nontolerated dose. Patients with AHF are frequently hypervolemic and hypertensive,[5] and may tolerate the vasodilation observed with BMS-986231 without clinically significant blood pressure reduction; this was borne out in the phase 2a study for BMS-986231.[20] Furthermore, although 15 μg/kg/min for 24 hours was inferred to be a nontolerated dose in healthy volunteers, the hypervolemia/hypertension associated with AHF may allow for higher dosing of BMS-986231 in this patient population. Of note, although the duration of infusion in the current study (24 or 48 hours) was substantially longer than that which was subsequently

employed in the phase 2a study (6 hours), hypotension seen in healthy volunteers in this study was typically observed within the first 2-4 hours of BMS-986231 administration. Indeed, the aforementioned phase 2a study also reported well-tolerated numerical (but not clinically significant) reductions in blood pressure versus placebo, consistent with the underlying volume overload in these patients with AHF. The phase 2a study also demonstrated statistically significant reductions in other hemodynamic parameters (PCWP, pulmonary arterial systolic and diastolic pressures, and right atrial pressure).[20]

Conclusions

This first-in-human study demonstrated an acceptable safety profile for BMS-986231 as a 24- or 48-hour continuous infusion in healthy volunteers. BMS-986231 demonstrated well-tolerated dose- and exposure-dependent decreases in blood pressure, consistent with its vasodilatory activity. In contrast, there were no clear dose-dependent increases in heart rate, and heart rate was generally similar with BMS-986231 and placebo. No cases of tachycardia or arrhythmia were reported. There was an increase in cardiac index with BMS-986231 versus placebo, consistent with preclinical studies demonstrating positive inotropy. Headache was alleviated by hydration, and no other significant drug-related AEs were noted.

These findings, together with subsequent phase 2a results, indicate that BMS-986231 may have a therapeutic profile in patients with AHF. A phase 2b study of BMS-986231 in patients with AHF is currently underway (clinicaltrials.gov, NCT03016325).

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Declaration of Conflicting Interests

D.C. was Executive Vice President of Development and Regulatory Affairs at Cardioxyl Pharmaceuticals at the time of the study. R.P.V. was Senior Director of Clinical Development at Cardioxyl Pharmaceuticals at the time of the study. K.L. has no conflicts of interest to disclose. J.T.G. is supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number K23NS085049, and was a site investigator for this trial. R.J.N. is supported by the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH) under Award Number UL1TR001117, and was the principal investigator for this trial. S.Y.F. was Chief Medical Officer of Cardioxyl Pharmaceuticals at the time of the study.

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Data Sharing

Bristol-Myers Squibb policy on data sharing may be found at https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html.

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Supporting Information

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