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Intravenous Infusion of the β(3)-Adrenergic Receptor Antagonist APD418 Improves Left Ventricular Systolic Function in Dogs with Systolic Heart Failure: β(3)-Adrenergic Receptor Antagonist in Heart Failure

Hani N. Sabbah

Kefei Zhang

Ramesh C. Gupta

Jiang Xu

Vinita Singh-Gupta

See next page for additional authors

Authors

Hani N. Sabbah, Kefei Zhang, Ramesh C. Gupta, Jiang Xu, Vinita Singh-Gupta, Michael Ma, Kathe Stauber, Nathalie Nguyen, and John Adams

Intravenous Infusion of the β_3-Adrenergic Receptor Antagonist APD418 Improves Left Ventricular Systolic Function in Dogs With Systolic Heart Failure

HANI N. SABBAH, PhD, FHFSA,[1] KEFEI ZHANG, MD,[1] RAMESH C. GUPTA, PhD,[1] JIANG XU, MD,[1] VINITA SINGH-GUPTA, PhD,[1] MICHAEL MA, MS,[2] KATHE STAUBER, PhD,[2] NATHALIE NGUYEN, PhD,[2] AND JOHN ADAMS, PhD[2]

Detroit, Michigan; and San Diego, California

ABSTRACT

Background: Unlike β_1- and β_2-adrenergic receptors (ARs), β_3-AR stimulation inhibits cardiac contractility and relaxation. In the failing left ventricular (LV) myocardium, β_3-ARs are upregulated, and can be maladaptive in the setting of decompensation by contributing to LV dysfunction. This study examined the effects of intravenous infusions of the β_3-AR antagonist APD418 on cardiovascular function and safety in dogs with systolic heart failure (HF).

Methods and Results: Three separate studies were performed in 21 dogs with coronary microembolization-induced HF (LV ejection fraction [LVEF] of approximately 35%). Studies 1 and 2 ($n = 7$ dogs each) were APD418 dose escalation studies (dosing range, 0.35−15.00 mg/kg/h) designed to identify an effective dose of APD418 to be used in study 3. Study 3, the sustained efficacy study, ($n = 7$ dogs) was a 6-hour constant intravenous infusion of APD418 at a dose of 4.224 mg/kg (0.70 mg/kg/h) measuring key hemodynamic endpoints (e.g., EF, cardiac output, the time velocity integral of the mitral inflow velocity waveform representing early filling to time-velocity integral representing left atrial contraction [Ei/Ai]). Studies 1 and 2 showed a dose-dependent increase of LVEF and Ei/Ai, the latter being an index of LV diastolic function. In study 3, infusion of APD418 over 6 hours increased LVEF from 31 ± 1% to 38 ± 1% ($P < .05$) and increased Ei/Ai from 3.4 ± 0.4 to 4.9 ± 0.5 ($P < .05$). Vehicle had no effect on the LVEF or Ei/Ai. In study 3, APD418 had no significant effects on the HR or the systemic blood pressure.

Conclusions: Intravenous infusions of APD418 in dogs with systolic HF elicit significant positive inotropic and lusitropic effects. These findings support the development of APD418 for the in-hospital treatment of patients with an acute exacerbation of chronic HF. (*J Cardiac Fail 2021;27:242−252*)

Key Words: Heart failure, left ventricular function, β-adrenergic receptors, β_3-adrenergic receptors, positive inotropic and lusitropic agents.

β-Adrenergic receptors (ARs) belong to the superfamily of membrane proteins known as G protein-coupled receptors. There are three recognized subtypes of β-ARs: β_1-ARs, β_2-ARs, and β_3-ARs. All three subtypes are expressed in cardiomyocytes but possess distinct intracellular signaling.[1] In the healthy heart, the β_1-AR is the predominant subtype and comprises nearly 70% of the β-ARs in the heart,[1,2] whereas β_2-ARs contribute approximately 20% of total β-ARs.[2] β_3-ARs are expressed at low levels in the healthy heart and seem to have a minimal impact on cardiac contractile function.[3,4] Stimulation of cardiac β_1-AR elicits a cyclic adenosine monophosphate- and calcium-dependent increase in contractility and has direct chronotropic effects via activation of pacemaker channels in the sinoatrial node resulting in increased heart rate (HR).[5,6] Selective stimulation of the β_2-AR also enhances cardiac contractility; however, the mechanism by which this occurs is not fully understood.[7] In contrast, β_3-ARs, activated by high concentrations of catecholamines, inhibit cardiac contraction and relaxation and have no direct chronotropic effects.[3,8−16] The intracellular signaling pathways modulated by the β_3-AR is an active area of research, but the negative inotropic effects observed are thought to result from coupling to the inhibitory G protein (G_i) and activation of the nitric oxide (NO)−cyclic guanosine monophosphate (cGMP) signaling pathway, resulting in decreased myofilament sensitivity to calcium.[9−11,15]

From the [1]Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Hospital, Detroit, Michigan and [2]Arena Pharmaceuticals, Inc., San Diego, California.

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Reprint requests: Hani N. Sabbah, PhD, FHFSA, Head, Cardiovascular Research, Henry Ford Hospital, 2799 West Grand Blvd, Detroit, MI 48202. Tel: (313) 916-7360; Fax: (313) 916-3001. E-mail: hsabbah1@hfhs.org

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A pivotal role for β-ARs in the progression of heart failure (HF) is characterized by increased levels of circulating catecholamines resulting from sustained activation of the sympathetic nervous system which over time becomes harmful as cardiac β-AR signaling becomes bioenergetically unfavorable and unresponsive owing to the downregulation and desensitization of β_1-ARs and β_2-Ars.[1,17,18] In contrast, expression of cardiac β_3-ARs is increased in several animal models of HF[9,19,20] and in the cardiomyocytes of patients with both ischemic and nonischemic HF.[13] Notably, β_3-ARs lack phosphorylation sites for the cyclic adenosine monophosphate-dependent protein kinase or β-adrenoceptor kinase required for the receptor desensitization process,[21] rendering them resistant to chronic catecholamine-induced desensitization.[22,23] In HF, increased β_3-AR activation is thought to serve as a protective mechanism to counterbalance the excessive activation of β_1/β_2-AR by catecholamines. In this context, myocardial β_3-ARs are hypothesized to serve as a physiological "brake" to suppress overstimulation of cardiac contractile function driven by sustained sympathetic activity.[15] Coordinated upregulation of the cardiac β_3-AR cognate G-protein subunit G_i in failing hearts[18,24] would further facilitate β_3-AR pathway−mediated suppression of cardiac contractile function. Experimental support for a direct role of β_3-AR in the failing heart comes from studies in dogs with pacing-induced systolic HF. Increased levels of endogenous catecholamines with chronic pacing correlated with depressed left ventricular (LV) contractility, and infusion of L-748,337, a selective β_3-AR antagonist, rapidly improved LV contraction and relaxation.[25] These findings suggest that the acute blockade of β_3-ARs can provide rapid relief of depressed cardiac contractile function in systolic HF.

Acute cardiac decompensation in patients with HF with reduced ejection fraction is a life-threatening condition where novel and safe therapies aimed at increasing cardiac contractility are needed. By selective inhibition of the β_3-AR, APD418 was designed to mitigate β_3-AR−mediated cardiodepression, and rapidly improve hemodynamic status. Furthermore, by targeting the selectively upregulated β_3-AR on cardiomyocytes, APD418 will enhance LV function and avoid the potentially deleterious effect on the systemic blood pressure or HR observed with current inotrope therapies. The objective of the present series of studies was to determine the effects of intravenous (i.v.) administration of APD418 on cardiovascular hemodynamic function in dogs with systolic HF produced by multiple sequential intracoronary microembolizations.

Methods

Animal Model

The dog model of intracoronary microembolization-induced systolic HF used in this study was previously described.[26] Twenty-one healthy, conditioned, class A dealer mongrel dogs, weighing between 19.9 and 30.6 kg, underwent serial intracoronary microembolizations performed 1 to 2 weeks apart, to produce HF. Coronary microembolizations were performed during cardiac catheterization under general anesthesia and sterile conditions. Embolizations were discontinued when the LV ejection fraction (LVEF), determined angiographically, was approximately 35%. Dogs were induced using a combination of i.v. injections of hydromorphone hydrochloride (0.22 mg/kg) and acepromazine (0.10−0.22 mg/kg). The plane of anesthesia was maintained throughout the study using 1% to 2% isoflurane. All dogs were maintained for at least 2 to 3 weeks after the last coronary microembolization before the study protocol was initiated. The study was approved by the Henry Ford Health System Institutional Animal Care and Use Committee and conformed to the National Institute of Health "Guide and Care for Use of Laboratory Animals" (NIH publication No. 85-23).

Study Protocols

Three separate studies were performed to achieve the desired objectives. All studies were performed under general anesthesia and sterile conditions as described elsewhere in this article.

Study 1, Dose Escalation I. A total of 7 dogs with HF were used in this study. All study measurements were performed during cardiac catheterization under general anesthesia and sterile conditions. After baseline hemodynamic, ventriculographic and echocardiographic measurements, vehicle (0.9% NaCl) was administered as a continuous constant i.v. infusion for 30 minutes. This was followed by 3 escalating doses of APD418 with each dose maintained for 30 minutes. The doses of APD418 were 1.4 mg/kg/h, 2.8 mg/kg/h, and 5.6 mg/kg/h. Doses were selected based on pharmacokinetic modeling targeting estimated exposures. At the end of each 30-minute infusion period, hemodynamic, ventriculographic, and echocardiographic measurements were taken. Venous blood samples were obtained at baseline and at 30 minutes after each dose. Blood samples were centrifuged at 3000 rpm for 10 minutes at 4 ˚C and plasma withdrawn and placed in cryostorage tubes and stored upright at −70 ˚C. Samples were used to determine the plasma concentration of APD418.

Study 2, Dose Escalation II. The purpose of this study was to identify a "no effect" dose of APD418 as well as a maximal dose of APD418 beyond which no further clinically significant incremental effects could be elicited. After baseline hemodynamic, ventriculographic, and echocardiographic measurements, APD418 or vehicle (5% dextrose; pH = 5.0) was administered as a continuous constant i.v. infusion. A total of 4 escalating doses of APD418 were administered and each dose maintained for 60 minutes. The doses of APD418 were 0.35 mg/kg/h, 5.6 mg/kg/h, 10.0 mg/kg/h, and 15.0 mg/kg/h. At the end of each 60-minute period, complete hemodynamic, ventriculographic, echocardiographic and myocardial oxygen consumption (MVO_2) measurements were made. Venous blood samples were obtained at baseline and at 60 minutes after each dose, were centrifuged at 3000 rpm for 10 minutes at 4 ˚C and

plasma extracted and placed in cryostorage tubes and stored upright at −70 ˚C. Samples were used to determine plasma concentration of APD418 and plasma levels of troponin-I (Tn-I). Ultra-sensitive dog cardiac Tn-I was measured by enzyme-linked immunosorbent assay using a commercially available kit.

Study 3, Single Dose, 6-Hour Infusion. The purpose of this study was to determine the temporal effects of a single dose constant infusion of APD418 over the course of 6 hours. In this study, each dog received a 6-hour constant i.v. infusion of APD418 (0.70 mg/kg/h) and a 6-hour constant i.v. infusion of APD418 vehicle (0.9% NaCl) with each infusion separated by at least 7 days. Hemodynamic, ventriculographic, echocardiographic, and MVO_2 measurements were made at pretreatment (baseline), before any drug or vehicle infusion, and repeated at 1, 2, 4, and 6 hours after initiating the infusions. Venous blood samples were obtained at baseline and at 1, 2, 4, and 6 hours after initiating each infusion. Samples were centrifuged at 3000 rpm for 10 minutes at 4 ˚C and plasma withdrawn and placed in cryostorage tubes and stored upright at −70 ˚C. Samples were used to determine plasma concentration of APD418.

Hemodynamic and Ventriculographic Measurements

All hemodynamic measurements were made during left and right heart catheterizations in anesthetized dogs. Cardiac catheterization was performed via a femoral artery and femoral vein approach. A 6F pigtail catheter and a 5F Swan-Ganz catheter were advanced into the LV cavity and main pulmonary artery, respectively, under fluoroscopic guidance and used to measure the phasic blood pressure. All data were recorded on and analyzed using an analog-to-digital system (Mennen Medical Ltd., Feasterville-Trevose, PA). The following parameters were evaluated in all dogs: (1) HR, (2) aortic pressure (AoP), (3) LV end-diastolic pressure (LVEDP), and (4) mean pulmonary artery pressure (mPAP). Left ventriculograms were obtained with the dog placed on its right side and recorded on digital media at 30 frames/s during the injection of 20 mL of contrast material (ISOVUE-300, Bracco Diagnostics, Inc., Princeton, NJ). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the left ventricle. LV end-systolic volume (ESV)) and LV end-diastolic volume (EDV) were calculated using the area−length method.[27] Premature beats and post-extra systolic beats were excluded from the analysis. The LVEF was calculated as the ratio of the difference of EDV and ESV to EDV times 100. The stroke volume (SV) was calculated as the difference between EDV and ESV. The cardiac output (CO) was calculated as the product of SV and HR. The systemic vascular resistance (SVR) was calculated as previously described.[28]

Two-Dimensional Echocardiographic and Doppler Measurements

Echocardiographic and Doppler studies were performed using a Philips EPIC-7 ultrasound system in conjunction with a Philips S5-1, 65 Hz, 10 cm transducer and recorded on digital media for off-line analysis. LV fractional area of shortening (FAS) was measured from 2-dimensional short axis views as the difference between end-diastolic and end-systolic areas divided by end-diastolic area times 100. Transmitral inflow velocity waveforms, measured using pulsed-wave Doppler echocardiography, were used to calculate indexes of LV diastolic function, namely, (1) the ratio of the mitral flow velocity integral during rapid early LV filling (Ei) to the flow velocity integral during atrial contraction (Ai), and (2) deceleration time of early mitral inflow velocity (DCT).[29]

MVO_2 Measurements

Measurements of MVO_2 were made in studies 2 and 3 as previously described.[30] Coronary artery blood flow velocity was measured using a Doppler flow velocity catheter (flow wire) placed in the proximal segment of the circumflex coronary artery. Blood flow was estimated by calculating the cross-sectional area of the circumflex coronary artery at the site of the catheter tip using coronary arteriograms. MVO_2 was determined as:

MVO_2 = (Total coronary blood flow) × (aorta to coronary sinus O_2 difference)

The oxygen content in the aorta and coronary sinus blood were measured using an AVOXimeter 1000 (A-VOX Systems, Inc., San Antonio, TX).

Measurement of APD418 Plasma Concentration

A liquid chromatography−tandem mass spectrometry bioanalytical method for the determination of APD418 in dog plasma (K_2EDTA) was performed by Charles River Lab (Montreal, Canada). Dog plasma samples were processed by protein precipitation, followed by dilution of the supernatant. Then, 10-μL aliquots of the processed samples were introduced for chromatographic separation using an Ascentis Express C18 (2.7 μm, 4.6 × 50 mm) column at a mobile phase flow rate of 1.00 mL/min. A gradient LC method was used. Mobile phase A was composed of 10 mM ammonium acetate:formic acid:trifluoroacetic acid (100:0.1:0.025 v/v/v); and mobile phase B was composed of methanol:formic acid:trifluoroacetic acid (100:0.1:0.025 v/v/v). Mass spectrometric detection was achieved with an AB Sciex API-4000 in positive electronic ionization mode using multiple reaction monitoring. The multiple reaction monitoring transition for APD418 and internal standard were 620 → 253 and 625 → 258, respectively. Quantification was performed using a linear regression ($1/x^2$ weighting) analysis generated from the peak area ratio of the analyte (APD418) over the internal standard (d_5-APD418) for the calibration standards. Reference standard APD418 mesylate was used for the preparation of calibration standards and QCs. The calibration curve ranges from 50.0 to 50,000.00 ng/mL.

β_3-AR Receptor Expression Study

Banked LV tissue from 7 normal dogs and 7 dogs with microembolization-induced HF stored at −70 ˚C, was used to confirm that LV tissue expression level of β_3-ARs receptors was upregulated in dogs with coronary microembolization-induced HF. None of these dogs were treated with APD418 and none received any HF therapies. The dogs, however, were of similar age and sex as the study dogs and those with HF had similar LVEF and duration of HF as the study dogs. Messenger RNA (mRNA) expression of β_3-AR and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (ADRB3: 5′-TTGGGTCTCATCATGGGCA-3′ (Forward), 5′-GCACAGAAGACGGCGGAA-3′ (Reverse); GAPDH: 5′-CCACTCCTCCACCTTTGAC-3′ (Forward), 5′- ACCC TGTTGCTGTAGCCA-3′(Reverse)) were measured in all dogs using real-time PCR. Protein levels of β_3-ARs (Novus Biologicals, LLC, Centennial, CO; Cat# NBP1-00716) and GAPDH (Fitzerald Industries International, Acton, MA, Cat# 10R-G109A) were measured in SDS extracts of LV tissue prepared from all dogs and subjected to Western blotting coupled with chemiluminescence detection and bands were quantified in densitometric units.

Chemicals: APD418

APD418 was provided as a powder by Arena Pharmaceuticals (San Diego, CA). The vehicle solution was 0.9% saline for studies 1 and 3, and 5% dextrose for study 2. The infusion mixture of vehicle and APD418 was prepared freshly just before daily administration.

Statistical Analysis

In all 3 studies, the primary LV systolic function endpoints were LVEF, SV, and CO, and the primary LV diastolic function endpoints were Ei/Ai and DCT. Studies 1 and 2 were designed as dose-ranging studies to identify a dose to be used in study 3. Study 3 was designed as the primary outcome study. Data analysis from study 3 was the analysis of primary interest.

Studies 1 and 2. Comparisons of hemodynamic, ventriculographic, echocardiographic, and Doppler measures were conducted using repeated measures analysis of variance with the alpha set at 0.05. If significance was attained, pairwise comparisons between baseline measures, vehicle and APD418 measures were made using the Student−Newman−Keuls test with a P value of less than .05 considered significant.

Study 3. Temporal comparisons for each intervention (vehicle or APD418) with respect to hemodynamic, ventriculographic, echocardiographic, and Doppler measures were made using repeated measures analysis of variance with the alpha set at 0.05. If significance was attained, pairwise comparisons between baseline measures and measures at 1, 2, 4, and 6 hours were made using the Student−Newman−Keuls test with a P value of less than .05 considered significant. Comparisons of the treatment effect change (difference between 6 hours and baseline for each intervention) was made using unpaired t-test with a P value of less than .05 considered significant. Comparisons between normal dogs and dogs with HF with respect to mRNA and protein β_3-ARs levels were made using an unpaired t-test with a P value of less than .05 considered significant. Data from all studies are reported as mean ± standard error of the mean.

Results

All 21 dogs in all 3 studies completed their respective protocols.

Regulation of β_3-AR expression

The mRNA expression of β_3-ARs normalized to GAPDH was increased 3.1-fold in coronary microembolization-induced HF dogs compared with normal dogs. Protein levels of β_3-ARs normalized to GAPDH were also markedly and significantly increased (approximately 9-fold) in HF dogs compared with normal dogs (2.053 ± 0.175 vs 0.222 ± 0.013; $P < .05$) (Fig. 1). These results confirm that β_3-ARs are significantly upregulated in dogs with coronary microembolization-induced HF compared with normal dogs.

Findings from Study 1: Preliminary Dose-Ranging Study

All hemodynamic, ventriculographic and echocardiographic/Doppler measures are shown in Table 1. When compared with vehicle, APD418 has no significant effects on HR, mean AoP (mAoP), or mPAP, and a modestly but significantly lowered LVEDP. The LV EDV was essentially unchanged during APD418 infusions, whereas the LV ESV decreased significantly and the LVEF and FAS increased

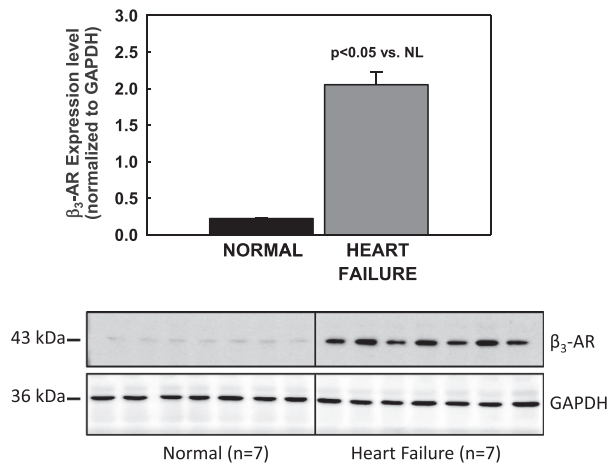

Fig. 1. Expression of β_3-adrenergic receptor (β_3-AR) in hearts from normal dogs and dogs with microembolization-induced heart failure. Western blots showing β_3-AR and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. Bar graph (mean ± standard error of the mean) shows the densitometric analysis of β_3-AR normalized to GAPDH in heart samples from failing dogs ($n = 7$) compared with normal (NL) dogs ($n = 7$). P values are based on unpaired t-test with a P value of less than .05 considered significant.

Table 1. Hemodynamic, Ventriculographic, Echocardiographic/Doppler Measurements Obtained at Baseline, at 30 Minutes After Vehicle Infusion, and at 30 Minutes After Infusion of Each of Three Doses of APD418 (Study 1)

	Baseline	Vehicle	APD418 (1.4 mg/kg/h)	APD418 (2.8 mg/kg/h)	APD418 (5.6 mg/kg/h)
LV EDV (mL)	62 ± 1	63 ± 1	62 ± 2	62 ± 2	61 ± 2
LV ESV (mL)	41 ± 1	41 ± 1	38 ± 1*,†	38 ± 1*,†	36 ± 1*,†
LVEF (%)	34 ± 1	35 ± 1	38 ± 1*,†	39 ± 1*,†	41 ± 1*,†
SV (mL)	21 ± 1	22 ± 1	23 ± 1*,†	24 ± 1*,†	25 ± 1*,†
CO (L/min)	1.70 ± 0.08	1.81 ± 0.07	1.94 ± 0.11	2.02 ± 0.08*,†	2.14 ± 0.08*,†
HR (beats/min)	81 ± 2	83 ± 1	83 ± 1	84 ± 1*	85 ± 1*
AoP*sys* (mm Hg)	88 ± 2	92 ± 2	92 ± 3	91 ± 4	90 ± 3
AoP*dias* (mm Hg)	61 ± 3	68 ± 3	67 ± 4	67 ± 5	66 ± 5
mAoP (mm Hg)	74 ± 2	79 ± 3	79 ± 4	79 ± 5	77 ± 4
LVEDP (mm Hg)	14 ± 0.6	15 ± 0.6	14 ± 0.8	13 ± 0.8†	12 ± 1.0*,†
SVR (dynes-s-cm^{-5})	3525 ± 257	3537 ± 152	3293 ± 205	3118 ± 183*,†	2895 ± 153*,†
mPAP (mm Hg)	16 ± 0.7	16 ± 0.6	16 ± 0.7	16 ± 0.5	15 ± 0.6
FAS (%)	34 ± 1	34 ± 1	37 ± 1*,†	40 ± 1*,†	41 ± 2*,†
Ei/Ai	3.1 ± 0.1	2.9 ± 0.3	3.6 ± 0.3*,†	3.7 ± 0.3*,†	4.3 ± 0.4*,†
DCT (msec)	98 ± 6	99 ± 9	119 ± 14	117 ± 11	133 ± 16*,†

AoPdias, diastolic aortic pressure; AoPsys, systolic aortic pressure; CO, cardiac output; DCT, deceleration time of the early rapid mitral inflow velocity waveform; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; Ei/Ai, time velocity integral of the mitral inflow velocity waveform representing early filling to time-velocity integral representing left atrial contraction; FAS, fractional area of shortening; HR, heart rate; LV, left ventricular; mAoP, mean aortic pressure; mPAP, mean pulmonary artery pressure; LVEDP, left ventricular end-diastolic pressure; SV, stroke volume; SVR, systemic vascular resistance.

*$P < .05$ vs baseline.

†$P < .05$ vs vehicle.

significantly in a dose-dependent manner, indicating an improvement of LV systolic function. The improvement in LVEF was accompanied by a dose-dependent increase in SV and CO (Table 1 and Fig. 2A). Infusion of APD418 caused a dose-dependent decrease in the SVR that reached significance at 2.8 mg/kg/h and 5.6 mg/kg/h. Furthermore, the infusion of APD418 resulted in a dose-dependent increase of Ei/Ai and DCT indicating improvement of LV diastolic function (Fig. 2A). No signs of arrhythmias were observed at any of the doses tested. The plasma samples at the 30-minute time points were collected and analyzed from each of the 7 dogs. The average dog plasma concentration results and statistics are summarized in Tables 2 and 3. These results show that APD418 plasma concentrations increased proportionately with an increased dose.

Findings from Study 2: Extension of the Dose-Ranging Study

All hemodynamic, ventriculographic, echocardiographic, and Doppler measures are shown in Table 4. At the lowest dose of 0.35 mg/kg/h, APD418 had no significant effects on the primary study end points of LVEF, CO, and Ei/Ai (Fig. 2B). Compared with vehicle, this dose of APD418 also had no significant effects on LV ESV, SV, FAS, HR, mAoP, LVEDP, mPAP, MVO_2, or Tn-I (Table 4 and Fig. 2B). APD418 at this dose, however, modestly but significantly decreased the SVR and increased the DCT. These data suggest that the dose of 0.35 mg/kg/h of APD418 is likely a no effect dose when viewed in terms of

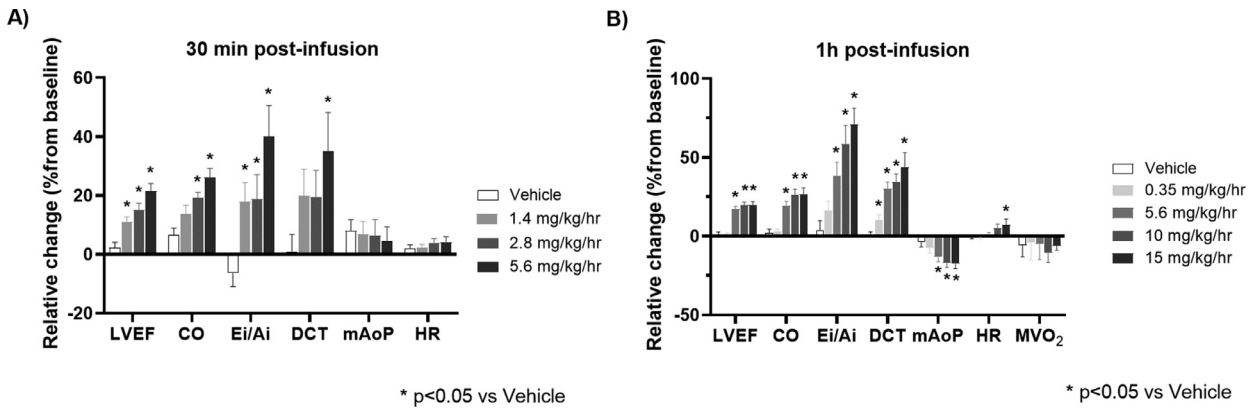

Fig. 2. APD418 dose-dependently improves systolic and diastolic function in a canine model of microembolization-induced heart failure. (A, B) Bar graphs (mean ± standard error of the mean) depicting dose-dependent increase in cardiac function after a 30-minute (A) or 60-minute (B) i.v. infusion of APD418. CO, cardiac output; DCT, deceleration time of mitral inflow velocity during early rapid LV filling; Ei/Ai, ratio of the mitral inflow velocity integral during rapid early LV filling (Ei) to the flow velocity integral during atrial contraction (Ai); HR, heart rate; LVEF, left ventricular ejection fraction; mAoP, mean aortic pressure; MVO_2, myocardial oxygen consumption. *P* values are based on a t-statistic for two means with a *P* value of less than .05 considered significant.

Table 2. Average Dog Plasma Concentrations Obtained at 30 or 60 Minutes After Infusion of Each Dose of APD418 (Studies 1 and 2)

Dose (mg/kg/h)	Plasma concentration (ng/mL)	*N*	%CV
Study 1 (30-min i.v. infusion of APD418)			
1.4	747 ± 21	7	7.5
2.8	1635 ± 44	7	7.1
5.6	3478 ± 58	7	4.4
Study 2 (60-min i.v. infusion of APD418)			
0.35	252 ± 15	7	15.6
5.6	3642 ± 195	7	14.2
10.0	7454 ± 390	7	13.9
15.0	12,178 ± 648	7	14.1

CV, coefficient of variation.

Table 3. Average Dog Plasma Concentrations Obtained at 1, 2, 3, 4, 5, and 6 Hours After Initiating APD418 Infusion (Study 3*)

Time (h)	Plasma Concentration (ng/mL)	*N*	%CV
1	485 ± 37	7	20.4
2	577 ± 50	7	22.7
3	633 ± 51	7	21.3
4	693 ± 61	7	23.2
5	717 ± 49	7	18.2
6	695 ± 60	7	22.8

Plasma concentration is represented as mean ± standard error of the mean.
Abbreviation as in Table 3.
*An i.v. infusion of APD418 at a constant dose of 0.70 mg/kg/h.

the absence of significant changes in measures of LV systolic function.

Higher doses of APD418 had no effects on EDV, but significantly decreased the ESV and significantly increased the LVEF, FAS, SV, and CO compared with vehicle (Table 4, Fig. 2B). There were only modest improvements among the 3 highest doses, favoring the highest dose. At 5.6 mg/kg/h and higher, there was a modest but significant decrease in the mAoP. Further decrease in the mAoP were observed at higher doses and accompanied by a small dose-dependent increase in the HR that reached significance at 15 mg/kg/h of APD418 compared with vehicle (Fig. 2B).

There was a significant dose-dependent increase in the indexes of LV diastolic function, namely, Ei/Ai and DCT (Fig. 2B). When compared with vehicle, the LVEDP did not change significantly at any of the higher doses of APD418 used. Compared with vehicle, the SVR decreased significantly at all higher doses of APD418 used. There were no significant differences in MVO_2 or mPAP at any dose tested. Plasma levels of Tn-I decreased significantly compared with the vehicle level. There were essentially no differences in the LV ESV, EF, CO, or FAS between APD418 doses of 10.0 and 15.0 mg/kg/h suggesting that these were the maximal effective doses, beyond which no further LV functional benefits are likely to be elicited. No signs of arrhythmias were observed at any dose tested. Plasma samples at the 1-hour time points were collected and analyzed from each of the 7 dogs. The average dog plasma concentration results and statistics are summarized in Table 2. These results show that APD418 plasma concentrations increased proportionately with the increased dose.

Findings From Study 3: Sustained Response and Primary Outcome Study

All hemodynamic, ventriculographic, echocardiographic, and Doppler measures are shown in Table 5. Vehicle infusion over 6 hours had no significant effects on the HR, LVEDP, mPAP, EDV, ESV, LVEF, CO, FAS, Ei/Ai, DCT, or MVO_2. The infusion of vehicle, however, caused modest decreases in the AoP and SVR that reached statistical significance. Compared with pretreatment measures, treatment with APD418 over 6 hours had no effects on the HR, EDV,

Table 4. Hemodynamic, Ventriculographic, and Echocardiographic/Doppler Measurements Obtained at Baseline, at 60 Minutes After Vehicle Infusion, and at 60 Minutes After Infusion of Each Dose of APD418 (Study 2)

	Baseline	Vehicle	APD418 (0.35 mg/kg/h)	APD418 (5.6 mg/kg/h)	APD418 (10.0 mg/kg/h)	APD418 (15.0 mg/kg/h)
LV EDV (mL)	52 ± 1	52 ± 1	52 ± 1	52 ± 1	52 ± 1	51 ± 1
LV ESV (mL)	35 ± 2	35 ± 2	35 ± 1	32 ± 1*,†	32 ± 1*,†	31 ± 1*,†
LVEF (%)	32 ± 1	33 ± 2	33 ± 2	38 ± 2*,†	39 ± 2*,†	39 ± 2*,†
SV (mL)	17 ± 1	17 ± 1	17 ± 1	20 ± 1*,†	20 ± 1*,†	20 ± 1*,†
CO (L/min)	1.36 ± 0.06	1.38 ± 0.05	1.39 ± 0.01	1.62 ± 0.08*,†	1.72 ± 0.10*,†	1.72 ± 0.10*,†
HR (beats/min)	81 ± 3	81 ± 2	81 ± 2	82 ± 2	85 ± 2	87 ± 2*,†
AoP*sys* (mm Hg)	96 ± 4	92 ± 4	92 ± 2	88 ± 2*	86 ± 2*	86 ± 2*
AoP*dias* (mm Hg)	65 ± 3	62 ± 3	59 ± 2*	53 ± 1*,†	52 ± 2*,†	54 ± 2*,†
mAoP (mm Hg)	76 ± 3	73 ± 3	70 ± 3	66 ± 2*,†	63 ± 2*,†	63 ± 2*,†
LVEDP (mm Hg)	13 ± 0.6	13 ± 0.4	12 ± 0.5	11 ± 0.5*	11 ± 0.7*	11 ± 0.3*
SVR (dynes-s-cm^{-5})	4527 ± 138	4266 ± 213	4069 ± 190*,†	3292 ± 139*,†	2981 ± 131*,†	2957 ± 143*,†
mPAP (mm Hg)	16 ± 0.5	15 ± 0.6	15 ± 0.8	15 ± 0.7	15 ± 0.8	15 ± 0.9
FAS (%)	33 ± 1	33 ± 1	34 ± 1	38 ± 2*,†	39 ± 2*,†	38 ± 2*,†
Ei/Ai	3.5 ± 0.5	3.5 ± 0.3	4.0 ± 0.5	4.7 ± 0.4*,†	5.5 ± 0.7*,†	5.8 ± 0.5*,†
DCT (msec)	123 ± 8	124 ± 8	136 ± 7*,†	159 ± 7*,†	164 ± 9*,†	175 ± 8*,†
MVO_2 (μmol/min)	131 ± 22	115 ± 10	118 ± 14	115 ± 12	110 ± 10	124 ± 21
Tn-I (ng/mL)	0.36 ± 0.03	0.34 ± 0.02	0.35 ± 0.02	0.18 ± 0.02*,†	0.18 ± 0.02*,†	0.19 ± 0.02*,†

MVO_2, myocardial oxygen consumption; Tn-I, troponin-I. All other abbreviations as in Table 1.
*$P < .05$ vs baseline.
†$P < .05$ vs vehicle.

Table 5. Hemodynamic, Ventriculographic, Echocardiographic, and Doppler Measurements Obtained Before Treatment (Baseline) and at 1, 2, 4, and 6 Hours After Initiating Vehicle or APD418 Infusion (Study 3)

	Before Treatment	1 Hour	2 Hours	4 Hours	6 Hours
Vehicle					
LV EDV (mL)	57 ± 5	57 ± 4	57 ± 5	57 ± 4	56 ± 4
LV ESV (mL)	39 ± 3	39 ± 3	39 ± 3	38 ± 3	38 ± 3
LVEF (%)	32 ± 1	32 ± 1	32 ± 1	32 ± 1	32 ± 1
SV (mL)	18 ± 1	19 ± 1	18 ± 2	18 ± 1	18 ± 1
CO (L/min)	1.47 ± 0.13	1.57 ± 0.14	1.47 ± 0.14	1.53 ± 0.14	1.49 ± 0.13
HR (beats/min)	82 ± 2	84 ± 3	80 ± 2	83 ± 3	82 ± 2
AoP*sys* (mm Hg)	94 ± 2	92 ± 2*	90 ± 3*	89 ± 2*	88 ± 2*
AoP*dias* (mm Hg)	63 ± 1	60 ± 1*	58 ± 1*	56 ± 2*	54 ± 1*
mAoP (mm Hg)	75 ± 1	72 ± 1*	70 ± 1*	67 ± 2*	66 ± 1*
LVEDP (mm Hg)	13 ± 1.0	13 ± 0.8	13 ± 0.9	12 ± 0.7	12 ± 0.8
SVR (dynes-s-cm^{-5})	4287 ± 467	3854 ± 412*	4045 ± 453*	3712 ± 398*	3755 ± 408*
mPAP (mm Hg)	15 ± 0.6	14 ± 0.5	14 ± 0.5	14 ± 0.4	14 ± 0.5
FAS (%)	32 ± 2	32 ± 1	32 ± 2	32 ± 1	32 ± 2
Ei/Ai	3.3 ± 0.3	3.5 ± 0.4	3.5 ± 0.3	3.4 ± 0.4	3.6 ± 0.4
DCT (msec)	134 ± 4	139 ± 3	137 ± 4	138 ± 5	139 ± 5
MVO_2 (μmol/min)	122 ± 20	114 ± 17	106 ± 10	116 ± 16	119 ± 19
APD418 (0.70 mg/kg/h)					
LV EDV (mL)	59 ± 4	59 ± 4	59 ± 4	58 ± 4	59 ± 4
LV ESV (mL)	40 ± 3	40 ± 3	39 ± 3	37 ± 3	37 ± 3
LVEF (%)	31 ± 1	33 ± 1*	35 ± 1*	36 ± 1*	38 ± 1*
SV (mL)	18 ± 1	19 ± 1*	21 ± 1*	21 ± 1*	22 ± 1*
CO (L/min)	1.46 ± 0.10	1.48 ± 0.10	1.65 ± 0.14*	1.70 ± 0.11*	1.77 ± 0.12*
HR (beats/min)	79 ± 2	77 ± 2	80 ± 2	82 ± 2	80 ± 3
AoP*sys* (mm Hg)	89 ± 1	92 ± 2	91 ± 1	89 ± 1	88 ± 2
AoP*dias* (mm Hg)	58 ± 2	63 ± 3	61 ± 2	59 ± 2	56 ± 1
mAoP (mm Hg)	70 ± 1	74 ± 3	71 ± 2	70 ± 1	67 ± 1
LVEDP (mm Hg)	13 ± 0.6	14 ± 0.6	12 ± 0.7*	11 ± 0.7*	12 ± 0.6*
SVR (dynes-s-cm^{-5})	3948 ± 317	4085 ± 310	3585 ± 292*	3368 ± 261*	3123 ± 229*
mPAP (mm Hg)	14 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.5	14 ± 0.5
FAS (%)	32 ± 1	33 ± 1	36 ± 1*	37 ± 2*	38 ± 2*
Ei/Ai	3.4 ± 0.4	3.6 ± 0.4	3.9 ± 0.5*	4.4 ± 0.5*	4.9 ± 0.5*
DCT (msec)	119 ± 7	128 ± 6	128 ± 6	133 ± 8*	144 ± 8*
MVO_2 (μmol/min)	138 ± 17	126 ± 10	134 ± 14	145 ± 13	151 ± 15

Abbreviations as in Tables 1 and 3.
*$P < .05$ vs before treatment.

mAoP, mPAP, or MVO_2 but decreased the LVEDP and SVR in a time-dependent manner. Treatment with APD418 tended to decrease the ESV and significantly increase the LVEF, FAS, SV, CO, Ei/Ai, and DCT in a time-dependent manner when compared with pretreatment.

Comparisons of the treatment effect Δ defined as the difference between measures at 6 hours and measures at baseline for each intervention (ie, vehicle or APD418) are shown in Table 6 and Fig. 3. There were no significant treatment effect differences with respect to the HR, LVEDP, mPAP, EDV, SVR, or MVO_2. Compared with vehicle, treatment with APD418 significantly increased the LVEF, CO, Ei/Ai, and DCT (Fig. 3). Systolic blood pressure, diastolic blood pressure, and mAoP were significantly higher during administration of APD418 compared with vehicle. These differences were driven primarily by the decrease in the AoP during vehicle administration, as discussed elsewhere in this article. No signs of arrhythmias were observed during the 6-hour infusion of APD418. Six plasma samples obtained hourly from 1 to 6 hours after dosing were analyzed for each dog. The average plasma concentration results and statistics are summarized in Table 3. Plasma concentrations correlated well with improved LV function observed during the 6-hour infusion with APD418 (Fig. 4). Correlation statistics were performed and a significant positive linear correlation between plasma concentration and improved LV function was observed ($R^2 = 0.9187$, $P = .0415$).

Table 6. Comparison of Treatment Effect Change (Difference Between 6 Hours and Before Treatment) for All Hemodynamic, Ventriculographic, Echocardiographic, and DOPPLER Measures Between Vehicle and APD418 (Study 3)

	Vehicle	APD418	*P* value
Δ LV EDV (mL)	−1.0 ± 0.6	0.0 ± 0.5	.225
Δ LV ESV (mL)	−1.1 ± 0.3	−3.6 ± 0.5	.001
Δ LVEF (%)	0.4 ± 0.5	6.4 ± 0.4	.0001
SV (mL)	0.1 ± 0.5	3.6 ± 0.4	.0001
Δ CO (L/min)	0.02 ± 0.04	0.31 ± 0.04	.0001
Δ HR (beats/min)	0.1 ± 1.3	1.0 ± 0.8	.570
Δ AoP*sys* (mm Hg)	−6.0 ± 1.4	−1.0 ± 1.6	.037
Δ AoP*dias* (mm Hg)	−8.6 ± 1.8	−1.7 ± 1.4	.011
Δ mAoP (mm Hg)	−8.6 ± 1.5	−2.9 ± 1.2	.012
Δ LVEDP (mm Hg)	−1.3 ± 0.7	−1.6 ± 0.2	.688
Δ SVR (dynes-s-cm^{-5})	−533 ± 112	−826 ± 131	.115
Δ mPAP (mm Hg)	−0.9 ± 0.3	−0.7 ± 0.4	.696
Δ FAS (%)	0.2 ± 0.4	6.3 ± 1.0	.0001
Δ Ei/Ai	0.34 ± 0.22	1.47 ± 0.19	.002
Δ DCT (ms)	4.3 ± 3.8	24.9 ± 4.9	.006
Δ MVO_2 (μmol/min)	−3 ± 6	13 ± 12	.256

Abbreviations as in Tables 1 and 3. *P* values are comparisons between vehicle and APD418 based on unpaired *t*-test.

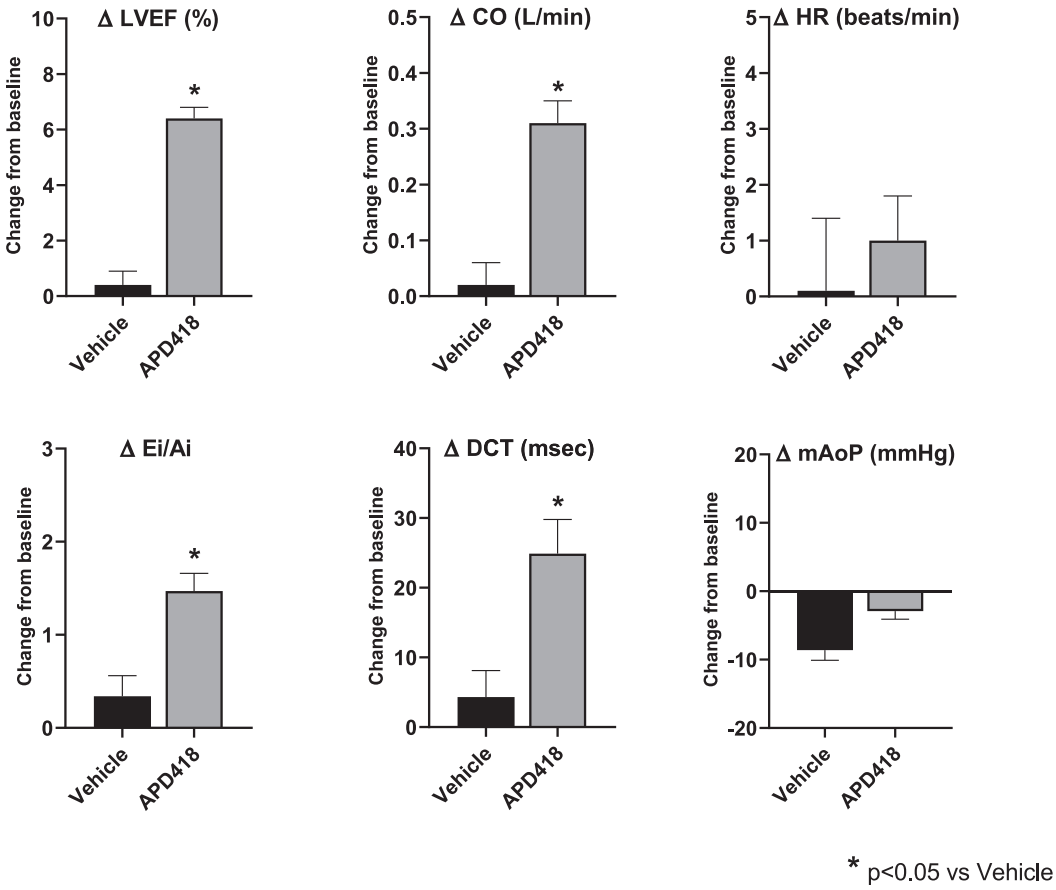

Fig. 3. Improvement in systolic and diastolic function with APD418 is sustained over a 6-hour treatment. (A) Bar graphs (mean ± standard error of the mean) depicting comparisons between treatment effect change between vehicle (black bars) and APD418 (grey bars) measures in study 3. Abbreviations as in Fig. 2. *P* values are based on a t-statistic for two means with a *P* values of less than .05 considered significant.

Discussion

The objective of the present studies was to determine the effects of i.v. administration of APD418 on cardiovascular function in dogs with systolic HF caused by multiple sequential intracoronary microembolizations. APD418 binds with high affinity to the human β_3-AR ($K_i = 8.2$ nM) displaying more than 400- to 600-fold selectivity over human β_1-AR and β_2-AR, respectively (Thuy-Anh Tran, 2015). Similar functional potency and selectivity of APD418 was also observed in Chinese Hamster Ovary cells expressing canine β_3-AR (Thuy-Anh Tran, 2015 and 2016). By comparison L-748,337, a first-generation β_3-AR antagonist, binds with high affinity to the human β_3-AR ($K_i = 4.0$ nM), but is only 90-fold and 45-fold selective over β_1-AR and β_2-AR, respectively.[31] Despite its suboptimal selectivity, acute i.v. administration of L-748,337 was shown to improve LV contraction and relaxation in a canine model of pacing-induced HF.[25] Further clinical development of this compound has not been pursued. SR59230A is another well-characterized compound previously described as a β_3-AR antagonist and commonly used in animal studies. However, further development has been hampered owing to its lack of selectivity for the human β_3-AR.[31,32] Despite their limitations, first-generation β_3-AR antagonists have been shown to improve LV and right ventricular function with either acute or chronic treatment in animal models of HF.[20,25,33,34] Additionally, mice with chronic β_3-AR deficiency showed improved contractile

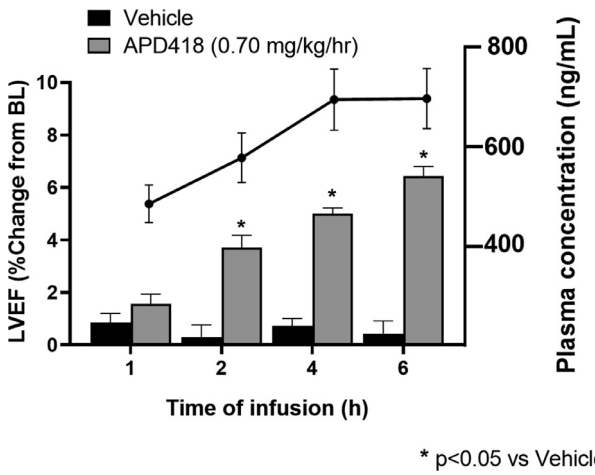

Fig. 4. Improvement in cardiac performance with APD418 correlates with plasma concentrations. Bar graph (mean ± standard error of the mean [SEM]) showing increase in left ventricular ejection fraction during a 6-hour i.v. infusion with continuous APD418 treatment (grey bars) and its correlation with increased plasma concentrations during the same period of time (line graph; mean ± SEM).

response to isoproterenol[12] and preserved myocyte function in a mouse model of isoproterenol-induced HF.[35] The potential therapeutic role of targeted β_3-AR modulation in acute and chronic HF is still being evaluated experimentally and clinically. However, our current approach was designed to evaluate the role of β_3-AR antagonism as an acute treatment in animals with systolic HF.

In isolated LV cardiomyocytes from dogs with rapid pacing-induced HF, stimulation with the selective β3-AR agonist BRL-37344 significantly decreased the cardiomyocyte contraction index dL/dt_{max} from 67.2 to 49.7 μm/s and significantly decreased the cardiomyocyte relengthening index, dR/dt_{max}, from 53.3 to 40.5 μm/s (36). This BRL-37344−induced negative inotropic response was abolished by pretreatment with APD418 (5×10^{-6} M).[36] These data indicate that APD418 is a β3-AR antagonist/blocker. The direct negative inotropic effect of β3-AR agonism on cardiac myocytes was confirmed in a recent study using human ventricular trabeculae from patients with HF with reduced ejection fraction, in which BRL-37344 at a concentration of 0.1 μM, significantly decreased the force of contraction by nearly 20%.[37] In this same study, inhibition of the β3-AR−mediated negative inotropy with APD418 at a concentration of 10 nM potentiated the force of contraction induced by norepinephrine by nearly 20%.[37] Our unpublished human receptor binding data strongly suggest that, at these concentrations, APD418 is not expected to interact with any other AR than β3-AR. These data provide further support that APD418 is an antagonist of the β3-AR. In other studies, acute i.v. administration of APD418 to dogs with normal heart function (prepacing) did not affect cardiac function (using LV end systolic pressure−volume relationship). However, after 4 weeks of pacing-induced HF, APD418 was administered and significantly improved cardiac performance in the same animals without changes in the HR or blood pressure.[36] Finally, previous studies also showed that L-748,337, another selective β_3-AR antagonist, prevented the β_3-AR−mediated negative inotropy in dogs with pacing-induced HF, supporting further the beneficial effect of selectively blocking β_3-AR in HF[9,25] and are consistent with the present study results using APD418.

In the present study, a highly validated canine model of microembolization-induced systolic HF was used to simulate HF with reduced ejection fraction in humans.[38−42] In both microembolization and pacing-induced canine models of HF, high levels of circulating catecholamines have been observed[25,26] and LV β_3-AR expression level is increased[9] (Fig. 1), recapitulating key observations found in LV myocardium of humans with HF.[13] In contrast with pacing-induced HF whereby LV functional recovery can occur slowly upon the cessation of rapid pacing, coronary microembolization leads to irreversible myocardial injury with slow progressive worsening of LV dysfunction, as seen in the human disease counterpart.[26,43] Two dose escalation protocols were conducted to delineate the subtherapeutic and maximum effect doses of APD418. At the lowest APD418 dose of 0.35 mg/kg/h, no significant improvement in measures of LV systolic function was observed suggesting that this may represent a no effect dose, at least from the perspective of LV systolic function. Higher doses starting at 1.4 mg/kg/h resulted in significant dose-dependent improvements in LV systolic and diastolic function, with further improvements observed up to 5.6 mg/kg/h. No further increases in cardiac function were observed above 10 mg/kg/h; however, a modest but significant decrease in the mAoP was observed at 5.6 mg/kg/h, with further decreases seen at higher doses. It is possible that, at these supratherapeutic doses of APD418, high plasma drug concentration results in the nonselective inhibition of β_1/β_2-ARs causing a reduction of mAoP accompanied by a reflex increase in the HR. At doses between 1.4 mg/kg/h and less than 5.6 mg/kg/h, APD418 infusion improved the LV systolic and diastolic function and was devoid of any chronotropic effects, systemic blood pressure−lowering effects, proarrhythmic effects, or increases in MVO_2 and plasma Tn-I. The absence of these undesirable effects at these doses are most likely owing to the selective blockade of the β_3-AR signaling pathway, which mainly involves modulation of the NO−cGMP−PKG signaling cascade, and does not directly alter cyclic adenosine monophosphate and intracellular Ca^{2+} levels, unlike conventional inotropic agents.[44] Numerous studies from multiple investigators have demonstrated that stimulation of β_3-AR activate G_i−NO−cGMP−PKG signaling pathway in the heart to induce a negative inotropic effect.[10,12,45,46] In fact, a previous study showed that blockade of NO synthase (with L-NAME) or G_i signaling (with pertussis toxin) inhibited a β_3-AR−mediated negative effect on the contractile function of cardiomyocytes isolated from dogs with pacing-induced HF.[9] Future mechanistic studies are planned in cardiomyocytes isolated from the heart of dogs with normal and decompensated heart function to confirm that APD418 inhibits the G_i−NO−cGMP−PKG signaling pathway in cardiomyocytes.

The primary translational finding of the present study is the sustained improvement in LV function seen during 6 hours of continuous infusion with APD418. Improved LV systolic and diastolic function were evidenced by an improvement in the indexes of LV systolic function, namely, LVEF and CO, as well as an improvement in the indexes of LV diastolic function, namely, Ei/Ai and DCT. This sustained effect correlated directly with the pharmacokinetic profile of i.v. APD418 infusion and suggests that continuous β_3-AR blockade provides sustained hemodynamic benefits. During the 6-hour infusion of APD418, no change in HR or mAoP were observed, indicating that APD418 does not act as a direct vasodilator and the decreased SVR observed in the dose-ranging studies is likely a compensatory response to a stable mAoP in the presence of a significant increase in CO. A significant decrease in mAoP and SVR was observed during vehicle infusion, but this result was most likely associated with the exposure to anesthesia during a 6-hour period. Despite the observed decrease in mAoP and SVR with vehicle, treatment with APD418 still resulted in significant improvements in cardiac function.

In chronic HF, β_3-AR upregulation and activation induce a negative inotropic effect that serves as a protective mechanism in response to the increased level of catecholamines. This physiological brake is an adaptive mechanism that potentially compensates for the sustained sympathetic overdrive.[15] Accordingly, other groups have proposed the use of β_3-AR agonists as a potential treatment for chronic HF. To date, the selective β_3-AR agonist (mirabegron), when evaluated in a small trial of patients with systolic HF (BEAT-HF) showed no improvement of LV function after 6 months of therapy.[47] However, patients with a baseline LVEF of less than 35% did show a positive treatment effect. In contrast with chronic HF, in patients with acute decompensated HF, the upregulated β_3-AR−mediated negative inotropic effect may be maladaptive, causing further contractile depression and exacerbating existing LV systolic dysfunction. Therefore, in this acute setting, removing the β_3-AR brake via selective inhibition with APD418, and remediating depressed contractile function could provide rapid hemodynamic improvement and promote cardiovascular and clinical recovery.

There are some limitations to the present study that merit consideration. Given the mechanism of action of APD418, this translational preclinical study was designed to primarily examine the short-term effects of APD418 on systolic LV function and not diastolic function. In retrospect, a more thorough analysis of LV diastolic function with measures such as pressure−volume relationships and measurements of longitudinal and radial strain would have been useful to better and more fully describe changes in LV diastolic function resulting from use of i.v. APD418. Another important limitation is the absence of data showing that APD418 actually targets downstream β_3-AR linked pathways. Such data would confirm that APD418 acts to increase contractility via inhibition of G_i−NO−cGMP−PKG signaling pathway in cardiomyocytes.

Finally, the results of the present series of studies, when viewed in concert, validate the rationale for advancing APD418 into clinical development and suggest β_3-AR antagonists as a promising therapeutic approach for the treatment of patients with acute systolic HF.

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