Online supplement

Group A: microembolization-induced ischemic cardiomyopathy model

Chronic left ventricular (LV) dysfunction and failure was produced by multiple sequential intracoronary embolizations with polystyrene latex microspheres. Coronary microembolizations were performed during cardiac catheterizations under general anesthesia and sterile conditions. Anesthesia was induced using a combination of intravenous injections of hydromorphone hydrochloride (0.22 mg/kg) and acepromazine (0.03 mg/kg). Plane of anesthesia was maintained throughout the study using 1–2% isoflurane. Left and right heart catheterization was performed via a femoral arteriotomy and venotomy. Microembolization was discontinued when LV ejection fraction (LVEF), determined angiographically, was approximately 30%. A period of 2 weeks was allowed after the final embolization to ensure that infarctions produced by the final microembolizations had completely healed before the study was undertaken.

BMS-986231 was provided by the sponsor as a powder and reconstituted with 15% Captisol® (Ligand, San Diego, CA, USA) in sterile water (pH 4) to give a stock solution of 10.65 mg/mL. Dosing solutions were prepared by dilution with 5% dextrose in water. Vehicle and BMS-986231 2 and 7 µg/kg/min were administered in a random order, with each infusion separated by at least 1 week. The 0.7 µg/kg/min dose was the final infusion administered. Animals were monitored for 1 hour post-infusion and hemodynamic, angiographic, and echocardiographic measurements were recorded at baseline until 5 hours after the start of infusion (Table S1).

Supplementary Table S1. Timings of hemodynamic, angiographic, and echocardiographic measurements in dogs with microembolization-induced HF (Group A, dose-escalation study)

		Minutes after infusion start											
	Predose	**30**	**60**	**120**	**180**	**240**	**245**	**250**	**255**	**260**	**270**	**285**	**300**
Pressures, ECG	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
CO, echo, Vgram	**X**		**X**	**X**		**X**							**X**
MVO_2*	**X**			**X**		**X**							
Blood samples for PK	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Blood samples for markers	**X**					**X**							

*Measured only with the 0.7 and 7 µg/kg/min infusions

CO, cardiac output; ECG, electrocardiogram; MVO_2, myocardial oxygen consumption; PK, pharmacokinetics

Hemodynamic and angiographic measurements

The following parameters were evaluated in all dogs: 1) arterial and LV pressures using catheter tip micromanometers (Millar Instruments, Houston, TX, USA); 2) peak rate of change of LV pressure during isovolumic contraction (peak +dP/dt) and relaxation (peak -dP/dt); 3) LV end-diastolic pressure (LVEDP); 4) cardiac output (CO). In addition, LV stroke volume (SV), systemic vascular resistance (SVR), and the time-constant of isovolumic LV relaxation (Tau) were all calculated based on a monoexponential model.

Left ventriculograms were performed with the dog placed on its right side and were recorded digitally at a rate of 30 frames/sec during a power injection of 15 mL of contrast material (ISOVUE-300, Bracco Diagnostics, Inc., Princeton, NJ, USA). Correction for image magnification was made using a radiopaque marker located on the distal end of the pigtail angiographic catheter. LV end-systolic and end-diastolic volumes were calculated from angiographic silhouettes using the area–length method. Premature beats and post-extrasystolic beats were excluded from the analysis. LV ejection fraction was calculated as the ratio of the difference of end-diastolic and end-systolic volumes to end-diastolic volume x100.

Echocardiographic and Doppler studies

Echocardiographic and Doppler studies were performed at all specified time points using a VIVID 7 Dimension ultrasound system (General Electric Healthcare, Chicago, IL, USA) in all dogs prior to ventriculogram recordings (Table S1). All echocardiographic measurements were made with the dog placed in the right lateral decubitus position and recorded on digital media for subsequent offline analysis. LV fractional area shortening (FAS) was measured from a short axis view at the level of the papillary muscles. LV major and minor semi-axes were measured to calculate LV end-diastolic circumferential wall stress (LVEDWS). Wall stress was calculated as follows: $\boldsymbol{Stress = Pb/h(1-h/2b)\ (1-hb/2a2)}$, where $\boldsymbol{P}$ is LV end-diastolic pressure, $\boldsymbol{a}$ is LV major semiaxis, $\boldsymbol{b}$ is LV minor semiaxis, and $\boldsymbol{h}$ is LV wall thickness.

Mitral inflow velocity was measured by pulsed-wave Doppler echocardiography to assess other LV diastolic function indexes. The velocity waveforms were used to calculate: 1) peak mitral flow velocity in early diastole (PE); 2) peak mitral inflow velocity during left atrial contraction (PA); 3) ratio of PE to PA; 4) time-velocity integral of the mitral inflow velocity waveform representing early filling (E); 5) time-velocity integral representing left atrial contraction (A); 6) ratio of Ei/Ai; and 7) deceleration time (DT) of the early rapid mitral inflow velocity waveform.

Aortic blood velocity was measured in the ascending aorta using flow Doppler. Aortic flow was calculated as the time integral of the aortic velocity waveform multiplied by aortic cross-sectional area.

Myocardial oxygen consumption measurements

Myocardial oxygen consumption (MVO_2) levels were measured at baseline and at 2 and 4 hours (with BMS-986231 0.7 and 7 µg/kg/min only). 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 distal to the first marginal branch. Blood flow was estimated by calculating the cross-sectional area of the CCA at the site of the catheter-tip using coronary arteriograms. Total LV coronary blood flow was estimated as twice that measured in the coronary artery. MVO_2 was determined as:

$$\boldsymbol{(MVO_2) = (\text{Total coronary blood flow}) \times (\text{aorta to coronary venous sinus } O_2 \text{ difference})}$$

Oxygen content in aorta and coronary venous sinus blood were measured using a hemoximeter (AVOXimeter 1000; A-VOX Systems, Inc.). The mechanical efficiency of the LV was calculated as the LV external mechanical power divided by the energy expenditure of the LV. The LV external power (watts) was calculated as the product of CO (L/min) and LV peak systolic pressure (LVPSP) using the equation:

$$\boldsymbol{\text{LV external power} = (CO)\ (10\text{-}6\ m^3/L)\ (LVPSP)\ (133.3\ \text{pascals/mmHg})\ /\ (60\ \text{sec/min})}$$

The energy expenditure of the LV was calculated from the MVO_2, which was converted from µmols/min to watts using the assumption that there are 0.008 J/µmol of O_2 consumed. Myocardial efficiency was calculated as follows:

$$\boldsymbol{\text{Mechanical Efficiency} = \text{LV Power} / \text{LV energy expenditure}}$$

Electrocardiographic measurements

Lead-II of the electrocardiogram was monitored throughout the study. The corrected QT interval was calculated based on the Bazett equation. If *de novo* ventricular arrhythmias developed during the study, the electrocardiogram was then recorded continuously. If at any time the arrhythmias became life-threatening and associated with hemodynamic collapse, drug infusion was stopped and the study terminated for that day; the animal was continually monitored after discontinuation to assess recovery rate.

Programmed ventricular stimulation

Programmed ventricular stimulation (PVS) was performed using an active fixation pacing lead advanced from the external jugular vein and positioned in the right ventricular apex (guided by fluoroscopy) and connected to a Vitality DS VR generator (Guidant T135) and a Zoom Latitude Programming System (Guidant 3120). The stimulation protocol included delivery of up to four extrastimuli at progressively shorter coupling intervals (starting at 350 msec, in steps of 10 msec). These extrastimuli were delivered after 8 ventricular paced beats with drive cycle length of 600–200 msec. PVS was initiated after a 2-hour IV infusion of BMS-986231 (7 µg/kg/min) and again after a 2-hour IV infusion of vehicle control. Animals received BMS-986231 or vehicle in a random order separated by at least 1 week. Each PVS was terminated when it provoked a sustained ventricular monomorphic tachycardia (SVT) or ventricular fibrillation (VF) lasting for >30 seconds.

Threshold data for SVT or VF were quantified based on a linear scaling factor, designating a low value of ‘1’ to S1 of 600 msec and S2 of 350 msec, and progressively increasing the score based on stimulation severity to the higher score of ‘24’ (designated for S5 of 200 msec). Beyond that, ‘Burst’ pacing (BP) was applied sequentially and finally, the highest score of ‘30’ assigned to incremental pacing (IP) of 300 msec for 6 sec duration. The scoring schema is shown in Table S2.

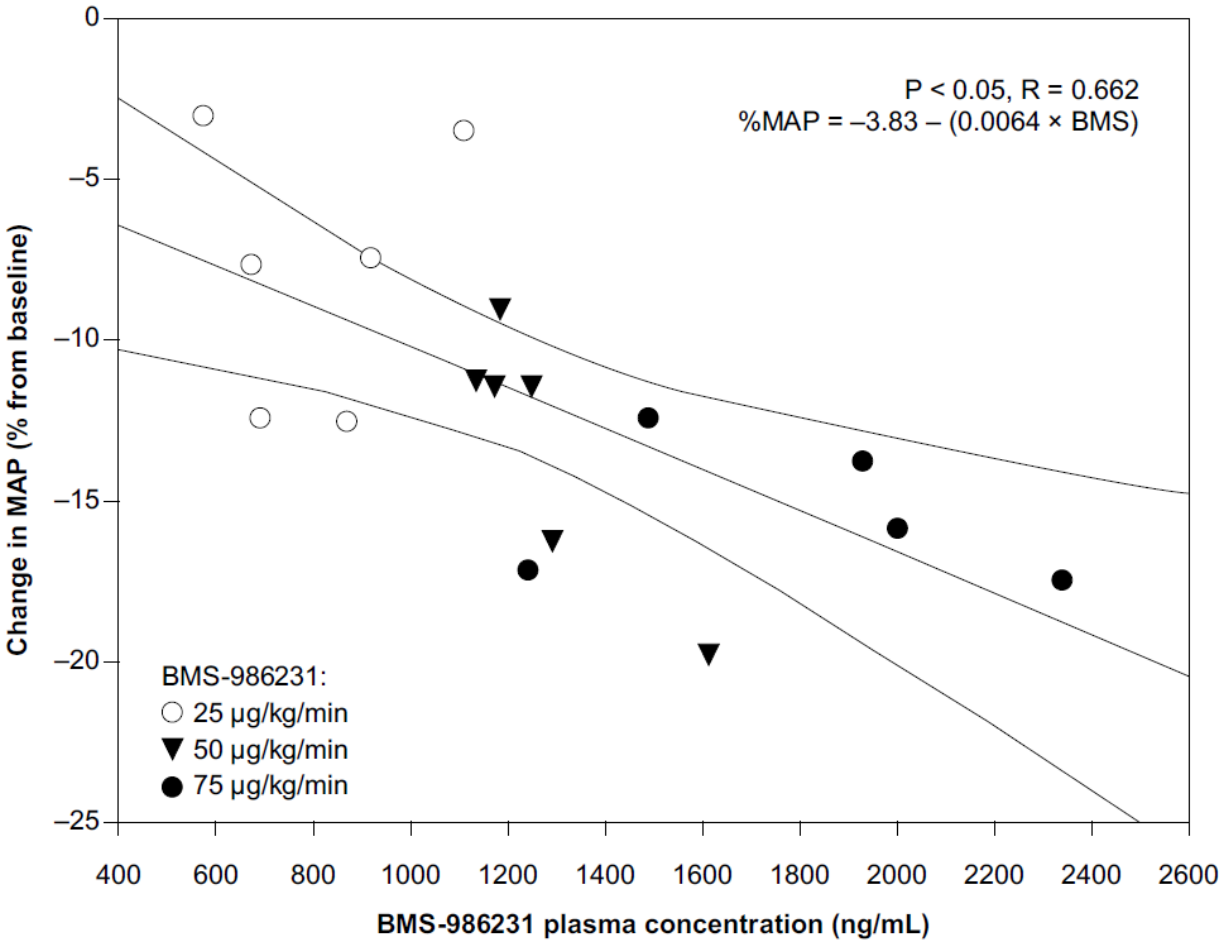
Supplementary Table S2. Threshold scoring schema for programmed ventricular stimulation

Threshold scoring schema							
S1 600	**S1 500**	**S1 400**	**S1 300**	**S1 250**	**S1 200**	**BP**	**IP**
S2 350-x=1	S2 300-x=5	S2 250-x=9	S2 200-x=13	S2 200-x=17	S2 200-x=21	300-x=25	300/10-2sec-x=28
S3 350-x=2	S3 300-x=6	S3 250-x=10	S3 200-x=14	S3 200-x=18	S3 200-x=22	250-x=26	300/10-4sec-x=29
S4 350-x=3	S4 300-x=7	S4 250-x=11	S4 200-x=15	S4 200-x=19	S4 200-x=23	200-x=27	300/10-6sec-x=30
S5 350-x=4	S5 300-x=8	S5 250-x=12	S5 200-x=16	S5 200-x=20	S5 200-x=24		

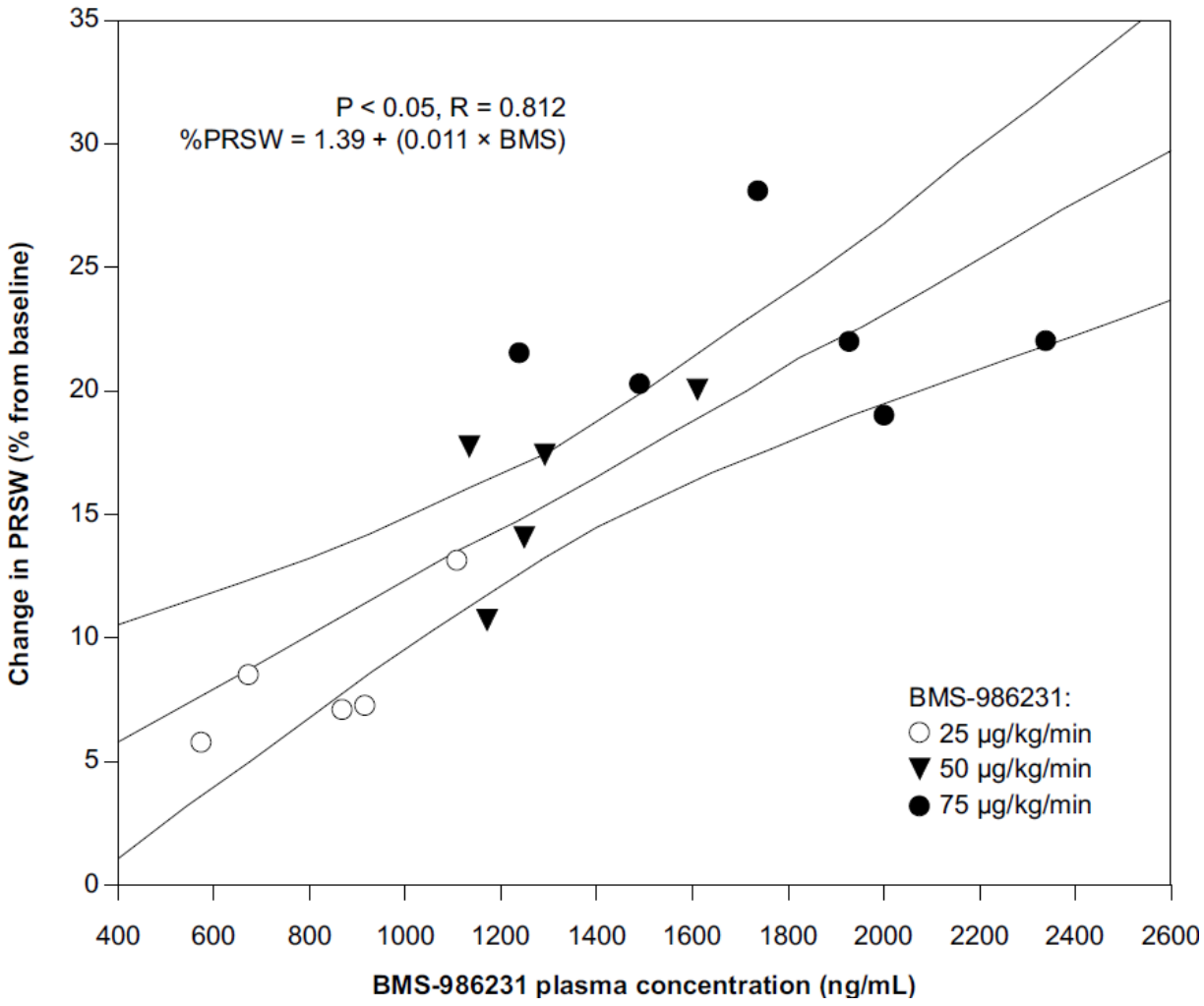
S1 600/500/400, etc., drive cycle length of 600, 500, 400 msec, etc.; BP, ‘burst’ pacing; IP, incremental pacing

Supplementary Figure S1. Scatterplots of individual plasma concentrations of BMS-986231 versus the associated individual relative (% change from baseline) changes in MAP (A) and PRSW (B) as measured at the end of the 3-hour continuous IV infusion of BMS-986231 25 µg/kg/min, 50 µg/kg/min, and 75 µg/kg/min in dogs with pacing-induced cardiomyopathy (n=6)

A.



B.



MAP, mean arterial pressure; PRSW, preload recruitable stroke work