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Comparison of the Cardiac Electrophysiology and General Toxicology of Two Formulations of Intravenous Amiodarone in Dogs

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Abstract Intravenous amiodarone (AIV) must be administered slowly after dilution to avoid hypotension, which is due to the cosolvents polysorbate 80 and benzyl alcohol used in its formulation. PM101 is a formulation of amiodarone devoid of these cosolvents, which enables bolus administration. We evaluated any potential toxicity or exaggerated adverse cardiac electrophysiologic effects of PM101 compared with AIV and control. Beagle dogs were treated with the human-equivalent amiodarone loading dose (2.14 mg/kg) with PM101 (bolus push) or AIV (10 min infusion in the toxicology study and bolus push in the electrophysiology study) followed by maintenance infusion (0.014 mg kg^{-1} min^{-1} through 6 h followed by 0.007 mg kg^{-1} min^{-1} through 14 days) or a control. General toxicology was assessed in conscious dogs over 14 days. Cardiac electrophysiology was assessed in a separate cohort of anesthetized dogs during the first 20 min of dosing. In the toxicology study, dosing in all animals in the AIV group was terminated within 17 min of initiation due to a severe hypersensitivity reaction. There were no acute adverse clinical signs in the PM101 or control groups. There were no significant effects on body weight or ECG parameters, and no adverse histomorphologic changes were seen in dogs that received PM101 or AIV. No significant exaggerated cardiac electrophysiologic effects of the approved doses PM101 or AIV were observed. PM101 may represent a formulation of intravenous amiodarone that could be administered rapidly without dilution in the setting of life-threatening cardiac arrhythmias.

Keywords Amiodarone · Cyclodextrin · Toxicology · Cardiac electrophysiology

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Introduction

Intravenous amiodarone is an effective treatment for ventricular and supraventricular cardiac arrhythmias [1–4]. Although useful in the acute treatment of a number of arrhythmias [1–5], clinically important hypotension during and after its administration is common, occurring in up to 26% of patients in these and other clinical studies [2, 6–8]. Intravenous amiodarone-induced hypotension often requires pressor therapy [6, 7] and deaths have been reported [6]. Intravenous amiodarone-induced hypotension is likely caused by the presence of the cosolvents polysorbate 80 and benzyl alcohol in the commercial formulation [9], and the risk of hypotension has led to limits on the rate of infusion during the loading phase of therapy [10]. In an effort to reduce the risk of hypotension during administration, several attempts have been made to develop alternative formulations of intravenous amiodarone devoid of polysorbate 80 and benzyl alcohols; however, none of them has been approved for human use [11–14].

PM101 is an intravenous formulation of amiodarone devoid of polysorbate 80 and benzyl alcohol that was developed to avoid the cosolvent-related issues associated with the conventional formulation of intravenous amiodarone. Animal testing has shown that administration of the human-equivalent loading dose of PM101 administered as a bolus push does not cause hypotension [15]. In order to enable evaluation of bolus push administration of PM101 in humans, it was necessary to assess the potential toxicity of PM101 when administered as an intravenous bolus push followed by maintenance infusion at the recommended human-equivalent dose. It was also necessary to rule out the potential that this more rapid rate of administration could produce exaggerated adverse cardiac electrophysiologic effects. The purpose of this study was to evaluate the toxicology and cardiac electrophysiology of PM101 compared with conventional intravenous amiodarone and placebo in dogs.

Materials and Methods

Ethics

All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The protocols were reviewed and approved by the Institutional Animal Care and use Committee of either CorDynamics (Chicago, IL) or Covance Laboratories (Vienna, VA), depending upon where the studies were performed.

Animals

Male or female beagle dogs between 7 and 15 months of age (6–12 kg) were obtained from Covance Research Products (Kalamazoo, MI). The dogs were housed in pens and kept on a 12 h light/dark cycle. Each dog received ~215 g/day of a 25% protein diet and each had free access to water.

Surgical Procedures: Toxicology Study

Animals ($n = 10$; 5 male and 5 female) were surgically instrumented with a jugular venous catheter (polyurethane) and subcutaneous vascular access port. All surgical procedures were completed at least 14 days prior to initiation of dosing. Surgical procedures were performed under injectable anesthesia. Animals were premedicated with buprenorphine (0.01 mg/kg IM) and midazolam (0.2 mg/kg IM) prior to transport to the surgery suite and were anesthetized with medetomidine (0.04 mg/kg IV). Additionally, injectable carprofen (4.4 mg/kg SC) was given as an anti-inflammatory agent and ceftiofur sodium (2.2 mg/kg IM) as an antibiotic at the time of surgical site preparation. Upon the completion of the surgical procedure, atipamezole (0.2 mg/kg IM) was administered to reverse the effects of the medetomidine. Animals were returned to the home cage after recovery.

Surgical Procedures: Cardiac Electrophysiology Study

Following induction of anesthesia (propofol, 5–6 mg/kg, i.v.), the dogs ($n = 7$ per group) were intubated. Anesthesia was maintained with isoflurane gas in 100% oxygen (1.5–2.5% in 1–2 l/min 100% oxygen). Morphine (0.5 mg/kg) was administered subcutaneously and bipivicaine (1 mg/kg of a 5 mg/ml solution) was infiltrated at the site of the chest incision (between the 2nd and 3rd or the 3rd and 4th ribs). The chest was opened and the heart was exposed. Two plunge electrodes were sutured to the epicardial right ventricular outflow tract and two plunge electrodes were sutured near the left atrial appendage. These electrodes were used for the measurement of atrial and ventricular effective refractory periods (AERP and VERP) and to record electrograms. A multipolar electrophysiology catheter (Bard Electrophysiology Division of C.R. Bard, Inc., Lowell, MA, USA) was positioned along the superior aspect of the tricuspid annulus in the anatomic vicinity of the bundle to measure atrioventricular (AV) nodal [i.e., atrial to His (AH)], and infranodal [i.e., His to ventricle (HV)] conduction intervals. A monophasic action potential duration catheter was placed into the left ventricle via the left carotid artery to record monophasic action potential duration time to 90% repolarization ($MAPD_{90}$). A solid-state pressure catheter was inserted into a femoral artery for measuring systemic arterial blood pressure. Hemodynamic, electrocardiographic, and electrophysiologic measurements were continuously monitored with the Notocord HEM v4.1.0.45 data capture system (Notocord, Croissy sur Seine, France). After completion of the surgical preparation, hemodynamic and electrophysiologic monitoring was initiated. Only dogs that exhibited normal and stable hemodynamic and electrophysiologic measurements prior to drug testing were used in the study.

Study Drugs

The commercial formulation of intravenous amiodarone (Amiodarone IV) was obtained from American Pharmaceutical Partners Inc. (Schaumburg, IL). This formulation contained amiodarone at a concentration of 50 mg/ml in a vehicle of polysorbate 80 (100 mg/ml) and benzyl alcohol (20.2 mg/ml) in water for injection with a pH of 4. PM101

was manufactured by HollisterSteir Laboratories (Spokane, WA). This stable formulation contained amiodarone (50 mg/ml) and sulfobutylether-7-beta-cyclodextrin (SBE7 betaCD; 225 mg/ml) in a 25 mM citrate buffer at a pH of 3.7. PM101 Vehicle was purchased from CyDex Pharmaceuticals (Lenexa, KS, USA) and contained the same ingredients as PM101 except that amiodarone was not included.

Dose Selection

The amiodarone dose chosen for this study was based on the FDA-approved US human loading dose of 150 mg followed by an infusion of 1 mg/min [10]. This equates to a loading dose of 2.14 mg/kg for a 70 kg patient and an infusion dose of 0.014 mg kg^{-1} min^{-1}.

Dose and Dosing Regimen: Toxicology Study

The loading dose of PM101 (2.14 mg/kg) was administered as a bolus push followed by an infusion (0.014 mg kg^{-1} min^{-1}; 1.8 mg/ml in 5% dextrose in water) for the next 6 h. Thereafter, a continuous maintenance infusion of 0.007 mg kg^{-1} min^{-1} (as 1.8 mg/ml in 5% dextrose in water) was administered through day 14. A control group (equivolume 5% dextrose in water) was dosed identically to the PM101 group. The Amiodarone IV group was dosed according to the approved human dosing schedule. Amiodarone IV (2.14 mg/kg) was administered as a loading infusion over 10 min from a 1.5 mg/ml solution in 5% dextrose in water. This was followed by an infusion (0.014 mg/kg/min; 1.8 mg/ml in 5% dextrose in water) for the next 6 h. Thereafter, a continuous maintenance infusion of 0.007 mg/kg/min (as 1.8 mg/ml in 5% dextrose in water) was administered through day 14.

Dose and Dosing Regimen: Cardiac Electrophysiology Study

All drugs were administered as a bolus intravenous injection followed by a continuous infusion, which was maintained throughout the duration of electrophysiologic testing. PM101 (2.14 mg/kg; $n = 7$) or Amiodarone IV (2.14 mg/kg; $n = 7$) was administered as an undiluted bolus push of the respective stock solution (50 mg/ml) followed by an infusion (0.014 mg/kg/min; 1.8 mg/ml in 5% dextrose in water). Control (5% dextrose in water; $n = 7$) or PM101 Vehicle (SBE7betaCD in 5% dextrose in water; $n = 7$) was administered as an equivolume bolus followed by infusion. Two additional groups of animals were dosed with a tenfold higher loading dose of either PM101 or Amiodarone IV (i.e., 21.4 mg/kg bolus push) followed by their respective standard infusion (0.014 mg/kg/min; 1.8 mg/ml in 5% dextrose in water). PM101 and Amiodarone IV were also administered as an undiluted bolus push alone without a subsequent standard infusion. This was done for the standard (2.14 mg/kg) and tenfold higher (21.4 mg/kg) loading dose of each PM101 ($n = 7$ per group) and Amiodarone IV ($n = 7$ per group).

Study Design: Toxicology

Each animal was observed twice daily (before and after noon) for mortality, abnormalities, and signs of pain or distress. During the dosing phase, each animal and the infusion system components were inspected twice daily to assess the system integrity. During the predose and dosing phases, cage-side observations were made for each animal. Body weight and food consumption were assessed weekly. Assessment of toxicity was done based on mortality, clinical observations, body weight, food consumption, ophthalmic and electrocardiogram evaluations, and clinical and anatomic pathology. For anatomic pathology, organs and tissues were harvested and preserved in 10% neutral-buffered formalin. Preserved tissues from each animal were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Study Design: Cardiac Electrophysiology

During each experiment, surface electrocardiograms and intracardiac electrograms were monitored continuously. Measurements were taken just before dosing and during the first 20 min after dosing. Intracardiac intervals were measured during sinus rhythm; refractory periods were evaluated at a pacing cycle length of 333 ms, corresponding to 180 beats per minute (BPM). Pacing was performed at twice the diastolic voltage threshold at a pulse width of 3 ms. AERP was measured during a train of eight atrial paced beats (S_1), followed by a single extra stimulus (S_2) which was progressively decremented until loss of atrial capture. The longest coupling interval (S_1S_2) that failed to capture the atrium was defined as the AERP. VERP was similarly measured during ventricular pacing, although constant S_1 was maintained via the aforementioned pacing cycle lengths. The longest coupling interval (S_1S_2) that failed to capture the ventricle was defined as the VERP. The sinus node recovery time (SNRT) was measured immediately after Wenckebach cycle length determination and defined as the longest pause from the last paced atrial depolarization to the first sinus return complex. Wenckebach periodicity was defined as the

longest interval (A_1A_1) that resulted in suprahisian AV block (A not followed by His) during decremental atrial pacing (S_1S_1).

Statistical Analysis

The effect of each drug or dosing regimen on cardiac electrophysiologic parameters was examined for statistical significance by one-way analysis of variance (ANOVA) followed by a Dunnett test for the comparison of each treatment to its respective baseline. A P value <0.05 was considered to be statistically significant.

Table 1 Summary of clinical signs

Category—sign	Control ($n = 10$)	PM101 ($n = 10$)	Amiodarone IV ($n = 10$)
Appearance			
Cage sore, left front interdigital	1		
Swollen, all paws			1
Swollen, ears			5
Swollen, muzzle			6
Swollen, periorbital			4
Swollen, right shoulder		1	
Behavior			
Hypoactive			6
Recumbent, Sternal			1
Discharge			
Vomitus, cloudy	1		
Vomitus, containing food		1	1
Vomitus, foamy	1		
Vomitus, white in color	1		
Excretion			
Mucoid feces	1		1
Nonformed feces	1	1	
Eyes			
Clear discharge, eyes			1
Respiration			
Labored			1
Panting			1
Skin and pelage			
Broken skin, right shoulder		1	
Red skin, ears			4
Red skin, ears, inside			3
Red skin, generalized			1
Warm to touch			1

No significant differences were noted (all P values were >0.05 by one-way ANOVA followed by Dunnett test)

Results

Toxicology

On day 1, the initial infusion in all of the animals in the Amiodarone IV group was terminated between 1 and 17 min after initiation of dosing due to adverse clinical signs, including hypoactivity, swollen ears, paws, and muzzle, and periorbital region, and reddened skin in the ears. These dogs were not dosed for the remainder of the day. These dogs were treated with diphenhydramine and prednisolone. Dosing was reinstated on day 2 at the maintenance infusion rate for the remainder of the dosing phase. An additional dose of diphenhydramine was administered to this group prior to initiation of dosing on day 2 but was not needed thereafter. During the remainder of the 14-day dosing phase, dogs in the Amiodarone IV group had no remarkable clinical signs of toxicity. As a result of this dosing interruption the Amiodarone IV group received a slightly lower total dose than the PM101 group.

Due to inflammation associated with the catheter, euthanization of one Amiodarone IV male and one PM101 female was performed on days 6 and 11, respectively. These events were not considered test article-related. All other animals survived to their scheduled necropsy.

Clinical signs in the control group consisted of one female with vomitus and abnormal feces (mucoid and/or nonformed) that may have been due to stress during dosing (Table 1). No remarkable treatment-related clinical signs were observed in the PM101 group.

There were no statistically significant differences between baseline and posttreatment body weights in the Amiodarone IV, PM101, or control group dogs (Table 2).

Table 2 Summary of body weight

Treatment phase	Mean ± SEM
Predose	
Control ($n = 10$)	8.55 ± 0.56
PM101 ($n = 10$)	8.40 ± 0.47
Amiodarone IV ($n = 10$)	8.58 ± 0.53
Week 1	
Control ($n = 10$)	8.54 ± 0.57
PM101 ($n = 10$)	8.35 ± 0.50
Amiodarone IV ($n = 9$)	8.22 ± 0.54
Week 2	
Control ($n = 10$)	8.54 ± 0.57
PM101 ($n = 9$)	8.56 ± 0.53
Amiodarone IV ($n = 9$)	8.31 ± 0.57

No significant differences were noted (all P values were >0.05 by one-way ANOVA followed by Dunnett test)

Electrocardiographic characteristics were also similar before and upon completion of treatment in the three groups (Table 3). The clinical pathology data were generally unremarkable and similar among the groups at each collection interval. No treatment-related ophthalmic lesions were noted. No adverse histomorphologic changes were seen in tissues examined from dogs that received PM101 or Amiodarone IV.

Cardiovascular Electrophysiology

The baseline cardiac electrophysiologic results are provided in Table 4. There were no significant differences at baseline for any parameter between the control group and the other treatment groups. Administration of control (5% dextrose in water) or PM101 Vehicle (SBE7betaCD in 5% dextrose in water) had no effect on any cardiac electrophysiologic parameter (Table 4). There were also no significant effects of the human-equivalent loading dose of PM101 (2.14 mg/kg) or Amiodarone IV (2.14 mg/kg) administered as a bolus push on any measured cardiovascular electrophysiologic parameters.

At the higher loading dose, both PM101 (21.4 mg/kg) and Amiodarone IV (21.4 mg/kg) produced a number of direct electrophysiologic effects consistent with what is known for amiodarone at this dose level (Table 4). The AH

Table 3 Summary of ECG parameters

Treatment group	HR (beats/min)	PR interval (s)	QRS interval (s)	QT interval (s)	QTcF interval (s)
Control					
Baseline ($n = 10$)	100.8 ± 5.4	0.082 ± 0.002	0.040 ± 0.000	0.198 ± 0.003	0.233 ± 0.007
Week 2 ($n = 10$)	119.0 ± 8.8	0.081 ± 0.004	0.041 ± 0.001	0.185 ± 0.005	0.229 ± 0.007
PM101					
Baseline ($n = 10$)	115.2 ± 6.1	0.078 ± 0.002	0.040 ± 0.000	0.185 ± 0.004	0.228 ± 0.004
Week 2 ($n = 9$)	126.0 ± 4.8	0.081 ± 0.004	0.040 ± 0.000	0.188 ± 0.005	0.240 ± 0.006
Amiodarone IV					
Baseline ($n = 10$)	113.4 ± 7.0	0.077 ± 0.003	0.042 ± 0.001	0.182 ± 0.004	0.225 ± 0.005
Week 2 ($n = 9$)	117.3 ± 5.6	0.080 ± 0.003	0.040 ± 0.000	0.184 ± 0.005	0.230 ± 0.004

Mean ± SEM. No significant differences were noted (all P values were >0.05 by one-way ANOVA followed by Dunnett test)

Table 4 Cardiac electrophysiologic parameters before and after treatment with PM101 bolus push followed by standard infusion

Treatment	Cardiac electrophysiologic parameters							
	AH	HV	WB	SNRT	TV	AERP	VERP	MAPD90
Control—baseline	101 ± 6	23.8 ± 3.3	180 ± 11	642 ± 79	3.1 ± 0.4	105 ± 5	153 ± 4	182 ± 9
Control—treatment	102 ± 5	22.3 ± 1.5	180 ± 9	623 ± 49	3.5 ± 0.7	106 ± 4	159 ± 10	167 ± 23
PM101 vehicle—baseline	102 ± 7	23.8 ± 1.2	184 ± 8	775 ± 86	3.5 ± 0.3	116 ± 5	156 ± 6	192 ± 18
PM101 vehicle—treatment	102 ± 9	23.7 ± 1.1	193 ± 11	822 ± 100	3.1 ± 0.2	120 ± 6	165 ± 8	198 ± 22
PM101 2.14 mg/kg—baseline	101 ± 5	21.1 ± 1.1	189 ± 6	654 ± 16	3.1 ± 0.2	120 ± 6	155 ± 4	196 ± 12
PM101 2.14 mg/kg—treatment	112 ± 5	21.8 ± 1.2	167 ± 9	715 ± 26	3.0 ± 0.2	120 ± 11	159 ± 5	192 ± 12
PM101 21.4 mg/kg—baseline	101 ± 7	23.8 ± 1.2	193 ± 13	627 ± 63	3.5 ± 0.3	117 ± 4	157 ± 2	188 ± 5
PM101 21.4 mg/kg—treatment	165 ± 33*	24.9 ± 0.8	87.0 ± 6*	3,617 ± 2,753	3.0 ± 0.2	148 ± 10*	158 ± 5	220 ± 4
Amiodarone IV 2.14 mg/kg—baseline	112 ± 8	23.3 ± 1.1	176 ± 10	690 ± 43	3.1 ± 0.3	107 ± 5	154 ± 6	201 ± 3
Amiodarone IV 2.14 mg/kg—treatment	117 ± 5	24.3 ± 1.5	143 ± 10	857 ± 26	3.2 ± 0.2	113 ± 6	160 ± 4	252 ± 3
Amiodarone IV 21.4 mg/kg—baseline	115 ± 8	23.4 ± 0.8	176 ± 8	624 ± 58	3.3 ± 0.2	103 ± 4	155 ± 3	193 ± 10
Amiodarone IV 21.4 mg/kg—baseline	181 ± 23*	23.3 ± 1.6	111 ± 18*	4,619 ± 2,466*	3.0 ± 0.1	135 ± 4*	162 ± 7	236 ± 11

Mean ± SEM. Each animal was paced at a pacing cycle length = 333 ms (180 BPM). There was no significant difference in any baseline value compared with the control baseline group

PM101 vehicle = SBE7betaCyclodextrin in 5% dextrose in water

AH atrial to His interval (ms); *HV* His to ventricle interval (ms); *WB* Wenckebach rate (BPM); *SNRT* sinus node recovery time (ms); *TV* diastolic threshold voltage (mV); *AERP* atrial effective refractory period (ms); *VERP* ventricular effective refractory period (ms); MAPD90, 90% monophasic action potential duration (ms); *PCL* pacing cycle length (ms)

* Significant change from baseline ($P < 0.05$; one-way ANOVA followed by Dunnett test)

interval was significantly increased in both groups indicating a slowing of AV nodal conduction (Fig. 1). Likewise, the cycle length corresponding to the first 1 appearance of Wenckebach block was significantly increased by PM101 (21.4 mg/kg) and Amiodarone IV (21.4 mg/kg), indicating relative decrease of AV nodal conduction (Fig. 2). The atrial effective refractory period (AERP) also significantly increased by at least 50% compared to pretreatment (Fig. 3).

The effects of PM101 and Amiodarone IV, administered as an undiluted bolus push alone without a subsequent standard infusion, were similar to that observed with administration of the bolus followed by the standard infusion (Table 5). Two animals each in the high-dose PM101 (21.4 mg/kg) and Amiodarone IV (21.4 mg/kg) bolus followed by standard infusion dose groups died or were declared in extremis due to protracted bradycardia or asystole. Two animals each in the high-dose PM101 (21.4 mg/kg) and Amiodarone IV (21.4 mg/kg) bolus alone (not followed by the standard infusion) dose groups died or were declared in extremis due to protracted bradycardia or asystole. One dog in the PM101 (2.14 mg/kg) bolus alone (not followed by the standard infusion) dosing group, during programmed electrical stimulation to interrogate refractoriness, experienced an extrastimulus-induced ventricular tachycardia that rapidly degraded into ventricular fibrillation for which no resuscitative efforts were made. Based on the cardiovascular condition of the animal and the temporal association with a short cycle length ventricular extrastimulus, the relationship of this VT/VF to PM101 exposure was deemed unlikely.

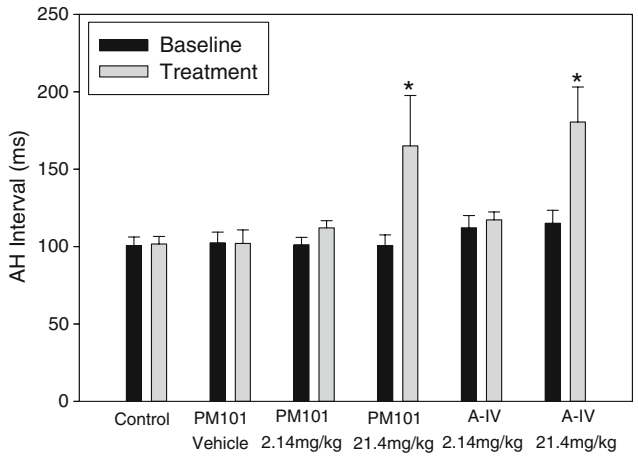

Fig. 1 Effect of treatment on atrial to His (*AH*) interval. * $P < 0.05$ compared to baseline

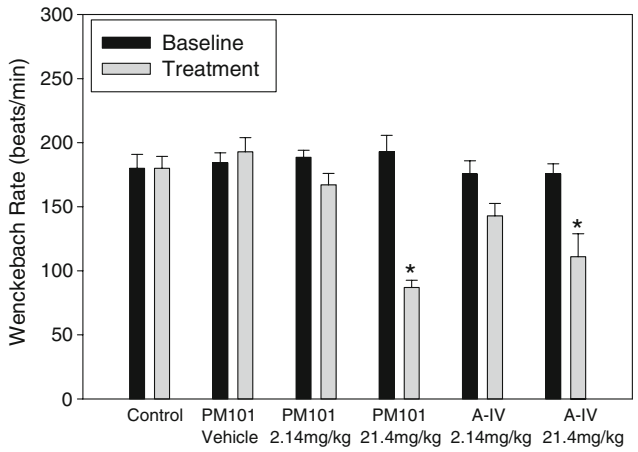

Fig. 2 Effect of treatment on Wenckebach rate. * $P < 0.05$ compared to baseline

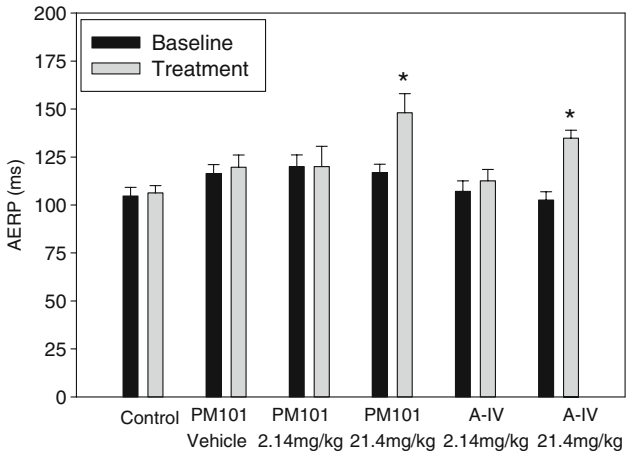

Fig. 3 Effect of treatment on atrial effective refractory period (*AERP*). * $P < 0.05$ compared to baseline

Discussion

PM101 is a new cosolvent-free formulation of intravenous amiodarone that is devoid of hemodynamic effects when the human-equivalent loading dose is given as a bolus push [15]. The primary purpose of the experiments presented in this report was to determine whether rapid administration of the human-equivalent loading dose of PM101 would unveil any exaggerated acute electrophysiologic effects not seen during the slower infusion of the loading dose of Amiodarone IV in dogs. A second purpose of this study was to assess the toxicologic profile of PM101 compared with Amiodarone IV and placebo.

In the present study, there were no notable electrophysiologic effects of the control agent (i.e., 5% dextrose in water) in this model. The acute administration of the PM101 vehicle (i.e., SBE7betaCD), at a dose equivalent to that found in PM101, also had no significant effect on any cardiac electrophysiologic parameter. PM101 exhibited

Table 5 Cardiac electrophysiologic parameters before and after treatment with PM101 bolus push alone without the standard infusion

Treatment	Cardiac electrophysiologic parameters							
	AH	HV	WB	SNRT	TV	AERP	VERP	MAPD90
Control—baseline	101 ± 6	23.8 ± 3.3	180 ± 11	642 ± 79	3.1 ± 0.4	105 ± 5	153 ± 4	182 ± 9
Control—treatment	102 ± 5	22.3 ± 1.5	180 ± 9	623 ± 49	3.5 ± 0.7	106 ± 4	159 ± 10	167 ± 23
PM101 vehicle—baseline	110 ± 11	22.1 ± 1.7	198 ± 15	779 ± 131	3.7 ± 0.4	107 ± 3	136 ± 5	176 ± 17
PM101 vehicle—treatment	111 ± 10	22.8 ± 1.2	200 ± 13	814 ± 199	3.5 ± 0.3	108 ± 4	139 ± 6	171 ± 22
PM101 2.14 mg/kg—baseline	109 ± 9	22.6 ± 1.0	165 ± 23	760 ± 60	2.9 ± 0.2	114 ± 6	139 ± 14	206 ± 4
PM101 2.14 mg/kg —treatment	123 ± 7	23.7 ± 1.4	155 ± 14	771 ± 52	2.9 ± 0.3	122 ± 7	140 ± 17	188 ± 10
PM101 21.4 mg/kg—baseline	128 ± 12	25.3 ± 1.7	158 ± 22	782 ± 52	3.4 ± 0.4	116 ± 12	155 ± 2	207 ± 8
PM101 21.4 mg/kg—treatment	184 ± 19*	27.6 ± 2.6	106 ± 7*	2,062 ± 1,275	3.2 ± 0.4	162 ± 17*	162 ± 3	251 ± 1
Amiodarone IV 2.14 mg/kg—baseline	105 ± 8	23.6 ± 1.7	185 ± 18	720 ± 88	2.7 ± 0.4	115 ± 11	161 ± 7	192 ± 13
Amiodarone IV 2.14 mg/kg—treatment	112 ± 4	23.0 ± 1.7	167 ± 13	729 ± 23	2.7 ± 0.2	128 ± 12	175 ± 10	214 ± 22
Amiodarone IV 21.4 mg/kg—baseline	101 ± 3	23.1 ± 1.4	180 ± 9	712 ± 70	3.4 ± 0.3	111 ± 6	162 ± 6	198 ± 8
Amiodarone IV 21.4 mg/kg—baseline	145 ± 8	24.0 ± 1.6	89.2 ± 5*	1,417 ± 483	3.1 ± 0.1	147 ± 7*	171 ± 4	208 ± 31

Mean ± SEM. Each animal was paced at a pacing cycle length = 333 ms (180 BPM). There was no significant difference in any baseline value compared with the control baseline group

PM101 vehicle = SBE7betaCyclodextrin in 5% dextrose in water

AH atrial to His interval (ms); *HV* His to ventricle interval (ms); *WB* Wenckebach rate (BPM); *SNRT* sinus node recovery time (ms); *TV* diastolic threshold voltage (mV); *AERP* atrial effective refractory period (ms); *VERP* ventricular effective refractory period (ms); MAPD90, 90% monophasic action potential duration (ms); *PCL* pacing cycle length (ms)

* Significant change from baseline ($P < 0.05$; one-way ANOVA followed by Dunnett test)

cardiac electrophysiologic properties that were qualitatively and quantitatively similar to Amiodarone IV, and consistent with the known acute electrophysiologic properties of Amiodarone IV [10]. At high doses, PM101 and Amiodarone IV each slowed AV nodal conduction and increased atrial refractoriness. There was no evidence of an exaggerated cardiac electrophysiologic response from PM101 administered as a bolus push compared to the equivalent dose of Amiodarone IV.

In the toxicology study, the initial response of the dogs treated with Amiodarone IV included hypoactivity, swollen ears, paws, and muzzle, and periorbital region, and red skin in the ears. These responses were severe enough to warrant halting the dosing to allow the animals to recover. These clinical signs in the Amiodarone IV group dogs were deemed to be attributed to histamine release induced by polysorbate 80, one of the vehicle components of Amiodarone IV [16]. The dogs were treated with an antihistamine and corticosteroid, and dosing was successfully restarted the next day after an additional dose of the antihistamine. By contrast, no unusual signs or symptoms were seen during dosing with PM101. No other physical or histologic changes were noted in either dosing group during the study.

In conclusion, rapid administration of the human-equivalent loading dose with PM101 did not cause any exaggerated electrophysiologic responses in anesthetized dogs, and no acute or chronic toxicological concerns were raised during 14 days of continuous dosing in dogs. Thus, PM101 may represent a formulation of intravenous amiodarone that could be administered rapidly without dilution enabling rapid achievement of therapeutic concentrations and antiarrhythmic effect in the setting of life-threatening cardiac arrhythmias.

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