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Myoblast Transfer in Ischemic Heart Failure: Effects on Rhythm Stability

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Skeletal myoblast (SM) implantation promotes recovery of myocardial function after ischemic injury. Clinical observations suggest an association of SM implantation and ventricular arrhythmias. Support for this link has been sought in animal studies, but none employing models of congestive heart failure. In a canine model of postinfarction congestive heart failure (CHF) we compared the frequency of rhythm disturbances using ambulatory electrocardiography monitoring following skeletal myoblast or saline (SAL) implantation. In 19 mongrel dogs ischemic injury and CHF were induced by intracoronary microsphere infusions. Direct intramyocardial injection of autologous skeletal myoblasts (ASM) ($2.7–8.3 \times 10^8$ cells) or SAL controls was administered to 11 and 8 dogs, respectively. Serial echocardiography and 24-h ambulatory electrocardiography were recorded at baseline (after CHF induction) and at 4 weeks and at 8–10 weeks after injection. Comparisons between groups of left ventricular ejection fraction (LVEF) and the frequency of ventricular arrhythmias, supraventricular arrhythmias, and measures of heart rate variability (HRV) were made at each of the three time points. LVEF increased from $41 \pm 6\%$ to $47 \pm 2\%$ ($p < 0.03$) in the ASM group, and did not change ($42 \pm 6\%$ to $40 \pm 2\%$, p = ns) in SAL. After injection, no differences were seen in the number of dogs demonstrating ventricular tachycardia ($n = 3$ vs. $n = 2$, p = ns) or frequent PVCs ($n = 3$ vs. $n = 3$, p = ns) in the ASM versus SAL groups, respectively. Significant changes were observed in a time–domain measure of HRV, standard deviation of normal-to-normal RR interval (in ms: 4 weeks 174 ± 95 vs. 242 ± 19; 8 weeks 174 ± 78 vs. 276 ± 78, ASM vs. SAL), but not in other time domain parameters. In this canine model of ischemic CHF, ASM implantation did not result in a significant increase in ventricular arrhythmias compared to controls animals. The potential for ASM implantation to affect time–domain parameters of HRV merits further study.

Key words: Congestive heart failure; Skeletal myoblasts; Canine model; Ventricular arrhythmia; Heart rate variability

INTRODUCTION

The potential of exogenous muscle progenitors to induce functional improvement in the postnecrotic ventricle has opened the door to novel strategies for myocardial repair (24,31). Ischemic injury is a common pathologic model to assess the effects of cell-based therapies, during both immediate and delayed ventricular recovery phases. Maladaptive postinfarction repair mechanisms lead to further myocyte loss, ventricular remodeling (36), and the clinical manifestation of congestive heart failure (CHF). The repopulation of myocardial fibrosis with skeletal myoblasts (SM) may bring on a partial reversal of post-MI left ventricular dysfunction is suggested by preclinical (2,3,14,37) and early clinical (13,20,23,34) observations.

Untoward effects may also arise from progenitor cell implantation (27,28). Myogenic progenitors may impact intrinsic electrical conduction through mechanisms such as reentry, enhanced automaticity, or triggered activity. While clinical manifestations of these mechanisms are unproven, support for proarrhythmogenic processes emerged with the first clinical series of autologous skeletal myoblast (ASM) implantation (20), in which 4 of 10 patients experienced ventricular tachycardia (VT) in the follow-up period, although subsequent reports describe a smaller incidence of VT (6,7,34) or none at all (13). The assessment of incremental risk is confounded by the high background incidence of symptomatic and asymptomatic arrhythmias in the eligible population (5,20).

The focus of most preclinical studies has principally

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been on efficacy and change in ventricular function, rather than dysrhythmias. Moreover, such studies have been mostly limited to mechanistic studies in small animals (1,21). The use of large animals for this purpose has been hampered by a paucity of models that effectively mimic CHF as well as the logistical challenge of arrhythmia surveillance. No studies have addressed the proarrhythmic potential of implanted cells in large animals models of heart failure. We therefore sought to determine the frequency of ventricular and other arrhythmias in a canine model of post-MI CHF following the intramyocardial administration of ASM in comparison to a saline (SAL)-injected control group. The primary objective of the study, to assess the efficacy ASM in this model, was reported (12). Here, we report an analysis of ambulatory electrocardiographic (AECG) findings from that study to further define the level of arrhythmia risk of ASM implantation.

MATERIALS AND METHODS

Study Design

The study design and protocol were described previously (12). Briefly, CHF was induced in 19 dogs. Four weeks later, ASM or SAL was administered by intramyocardial injection, the first 8 animals surgically (5 ASM, 3 saline) and the next 11 endovascularly (6 ASM, 5 saline), thus leading to four treatment groups. Hemodynamic, echocardographic, and AECG evaluations were made at baseline (CHF), and at 4 weeks and 8–10 weeks after intramyocardial injections. The study was approved by the Institutional Animal Care and Use Committee of the College of Physicians & Surgeons of Columbia University and conducted at the Animal Research Facility of Columbia University School of Medicine. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (15).

CHF Induction: Animal Preparation and Management

Mongrel dogs (27–35 kg) were instrumented to induce heart failure and to evaluate parameters of left ventricular function as previously described (12). Through a left thoracotomy, indwelling catheters were placed in the LV, aorta, and left anterior descending coronary artery, and pressure gauges and sonomicrometer crystals in the left ventricle. Four ECG wires were implanted in the anterior chest wall for AECG monitoring. All catheters and wires were externalized through the dog's back.

CHF, defined as LV end-diastolic pressure (LVEDP) ≥16 mmHg and maximal reduction in LV systolic pressure per second (LV dP/dt_{max}) of ≥ 25–30%, was induced by daily infusion through the LAD catheter of 25,000–50,000 polystyrene microspheres (98–115 μm diameter; Bangs Laboratories, Fishers, IN) (30).

Once in CHF, animals were maintained on a low-sodium diet and given furosemide as needed. Angiotensin converting enzyme inhibitors and beta blockers were not administered. Free ambulatory access was permitted within individual $4' \times 6'$ cages.

Autologous Skeletal Myoblast Preparation

After the development of CHF, skeletal muscle biopsies were taken as described (12). Isolated ASM were cultured in proprietary media (Bioheart, Inc. Weston, FL) that included fetal bovine serum. At each passage and at final harvest, staining for desmin (DAKO Monoclonal Mouse Anti Human Desmin Clone D33, Dako Cytomation, Carpinteria, CA) was performed. Cell number and viability were determined by trypan blue dye exclusion. On release, cells were suspended in transport medium (Hypothermosol FRS, Mediatech, Inc, Herndon, VA) to a concentration of 50 million cells/ml and sent to the Animal Research Facility. Using the above methods, fetal bovine serum was not detectable in the final cell products from a series of volunteers (data on file, Bioheart, Inc.).

Study Intervention

Approximately 8–9 weeks after initial surgery, intramyocardial injections of either ASM ($n = 11$) or SAL ($n = 8$) were performed. In that an objective of the study was to compare different cell delivery methods (12), both ASM and SAL injections were performed either surgically or percutaneously. For surgical administration, the area of infarction was exposed through a second thoracotomy. ASM or SAL was delivered via a 25-gauge needle in 0.25 ml (12.5×10^6 cells) aliquots, distributing the total dose evenly over the infarct and its margins. Percutaneous injections were accomplished through a needle-based catheter (8 Fr, MyoCath™, Bioheart, Inc., Weston, FL), introduced into the left ventricle via a femoral artery. Prior to catheter-based injection, akinetic target regions were identified from radiocontrast left ventriculography, the silhouettes of which were used for real-time fluoroscopic guidance. The deflectable distal tip of this device enables access to most regions of the endocardium and an extendable 25-gauge needle provides a portal for intramyocardial delivery (32). Injections of either cell suspension or SAL were distributed over akinetic and border zone regions. Lidocaine (in 1 mg/kg boluses) was administered as indicated for frequent premature ventricular beats or ventricular tachycardia.

Characteristics of intramyocardial injections are displayed in Table 1. In the ASM arm, five dogs received surgical and six catheter injections of $4.6 \pm 1.8 \times 10^8$ cells (range 2.7×10^8 to 8.3×10^8) ASM. Higher numbers implanted in the percutaneous group reflect a higher cell yield. In the SAL group, injections were car-

Table 1. Treatment Groups

Group	Injection	*N*	Cells Injected (Range)	% Desmin	Volume Injected (ml)	No. of Injections
ASM	surgical	5	$3.6 \pm 0.9 \times 10^8$	50 (40–70)	4.2 ± 0.7	16.4 ± 2.9
	percutaneous	6	$5.5 \pm 2.3 \times 10^8$	55 (50–85)	6.0 ± 1.9	23.0 ± 7.0
SAL	surgical	3	NA	NA	4.0 ± 1.0	16.3 ± 0.6
	percutaneous	5	NA	NA	5.0 ± 0.9	20.0 ± 3.4

Treatment characteristics of ASM and SAL groups. Within each group, ASM or SAL was given by two different methods: surgical (transepicardial) or percutaneous (transendocardial). One animal, receiving neither, was placed in the SHAM group. Desmin ranges are shown in parentheses. NA, not applicable.

ried out surgically in three animals and percutaneously in five animals. An attempt was made to match the numbers of injections in the SAL animals to those in the ASM group.

Hemodynamics and Left Ventricular Function

Hemodynamic parameters were measured in a conscious, recumbent nonsedated state. Parasternal two-dimensional echocardiographic images (SONOS 5500, Agilent Technologies, Andover, MA) were obtained with a 3.5-MHz probe at $t = 0$, 4, and 8 weeks. Left ventricular dimensions were measured at the basal and midpapillary levels using integrated software (11,17). Global ejection fraction (EF) was estimated from these measurements according to the methods of Dubroff (17).

Arrhythmia Assessment

Ambulatory ECGs (AECGs) were obtained at three intervals: baseline (CHF), 4 weeks postintervention, and 8–10 weeks postintervention. Implanted ECG wires were attached to magnetic tape recorders (E1HD1806FR, Marquette Electronics Inc., Milwaukee WI). Twenty-four-hour recordings (Sony HF90, Sony Electronics Inc., New Jersey, USA) were analyzed with a Century Scanner (Biomedical Systems, St. Louis, MO) and read by one of us (P.L.), blinded to treatment group. Premature ventricular contractions (PVCs), ventricular tachycardia (VT: 4 or more ventricular beats of average rate >110/min), supraventricular tachycardia (SVT: 4 or more ventricular beats of average rate >110/min), sinus bradycardia (rate below 40 bpm), and sinus pauses (>3 s) were tabulated. Frequent PVCs were defined as >30 on any single hour. Parameters of heart rate variability (HRV) were measured, including mean RR interval, standard deviation of normal-to-normal RR intervals (SDNNRR), root mean square of successive NN differences (RMSSD), and percentage of adjacent NN intervals in a 24-h recording, which differ by at least 50 ms (pNN50).

Unmonitored sudden deaths were considered arrhythmia related in the absence of other identifiable causes. Postmortem examination was performed on all animals.

Statistical Analysis

Continuous data (expressed as mean ± SD) were compared by Student's *t*-test and categorical data by Fisher's Exact test. The primary statistical analysis compared the frequency of VT on post-baseline AECGs between treatment groups. Secondary analyses compared intergroup frequencies of combined VT and sudden death, PVCs, bradyarrhythmias, and measures of heart rate variability. SPSS Exact Tests (SPSS, Chicago, IL) were used for statistical comparisons.

RESULTS

Study Flow and Animal Attrition

Nineteen dogs (11 in the ASM group and 8 in the SAL group) underwent baseline hemodynamic, echocardiographic, and AECG recordings after the induction of CHF ($t = 0$). Five dogs died prior to study completion: one due to refractory VT during catheter ASM injection; two suddenly (at 8 h after surgical SAL injection and at 5 weeks following catheter ASM injection); and two animals were euthanized due to advanced CHF (both in the catheter SAL group, at 6 and 9 weeks) (Fig. 1).

Hemodynamics and LV Function

Hemodynamic data are presented in Table 2. The development of CHF in both groups was associated with comparable changes in LVEDP and LV dP/dt_{max}, as required by the protocol, were observed in both groups. At CHF baseline ($t = 0$), HR, MAP, LVEDP, and LVSP were not different between groups. However, after intramyocardial injections LVEDP was lower (19.8 ± 3.8 vs. 25.0 ± 3.6 mmHg, $p < 0.05$) and LV dP/dt_{max} higher (2511.7 ± 137.7 vs. 2246.1 ± 123.3 mmHg/s, $p < 0.05$) in the ASM group at 4 weeks; the difference in LV dP/dt_{max} persisted at 8 weeks (2582.5 ± 135.4 vs. 2311.2 ± 202.9 mmHg/s, $p < 0.05$).

LVEF rose from baseline to 8 weeks (Fig. 2) in ani-

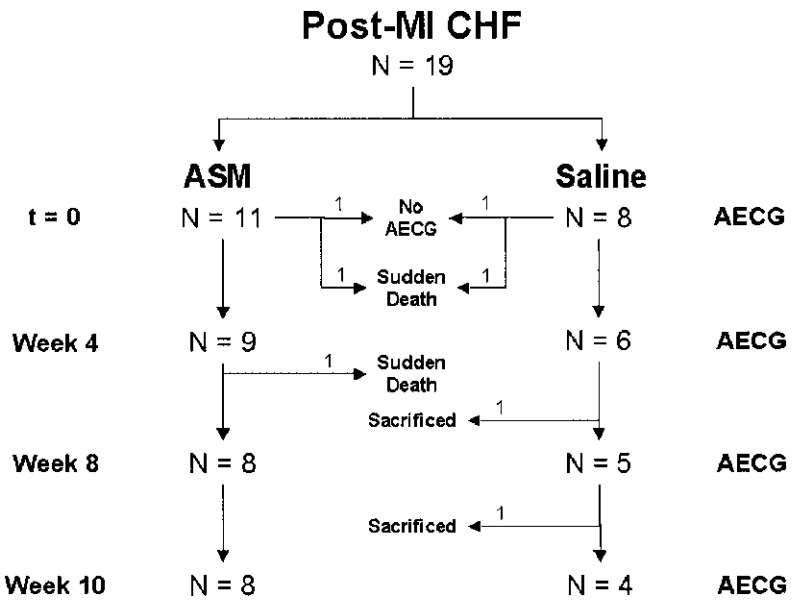

Figure 1. Nineteen dogs with post-myocardial infarction congestive heart failure (Post-MI CHF) assigned to group autologous skeletal myoblast injection (ASM, $n = 11$) or SAL group ($n = 9$). Ambulatory ECGs (AECG) were obtained following the induction of CHF ($t = 0$) and at 4, 8, and 10 weeks after injection.

mals injected with ASM ($41 \pm 6\%$ to $47 \pm 2\%$ ($p < 0.03$), whereas no change was seen following SAL injections ($42 \pm 6\%$ to $40 \pm 2\%$, $p =$ ns). Comparable increases in LVEF after ASM implantation were seen in both surgical and percutaneous subgroups (data not presented).

Arrhythmias and Events

Ambulatory ECG (AECG) data are presented in Table 3. Compared to baseline values, no significant differences were observed in the incidence of PVCs or VT at follow-up in either ASM or SAL, although the number of events and their ranges were fairly wide. Thus, two animals developed frequent PVCs and VT at 4 weeks, and four animals developed either frequent PVCs or VT at week 8. In the SAL group, serious ventricular arrhythmias were observed in three animals at baseline, all with VT. Fewer events were noted in follow-up, with a single animal developing ventricular arrhythmias at both 4 and 8–10 weeks. One dog in the SAL group with frequent

Table 2. Hemodynamics

Group	Time (Weeks)	HR (bpm)	MAP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)	LV dP/dt_{max} (mmHg/s)
ASM	pre-CHF	99.0 ± 17.1	103.2 ± 5.5	131.8 ± 6.8	10.8 ± 1.3	2988.7 ± 183.7
	0	102.0 ± 12.5	92.6 ± 4.6	113.1 ± 6.0	19.9 ± 2.0†	2250.4 ± 141.6
	4	98.4 ± 11.1	98.1 ± 3.0	121.2 ± 7.2	19.8 ± 3.8*	2511.7 ± 137.7*
	8	96.6 ± 16.0	98.7 ± 4.4	124.0 ± 4.8*	18.9 ± 2.5	2582.5 ± 135.4*
SAL	pre-CHF	97.2 ± 16.5	102.6 ± 3.1	129.2 ± 5.9	11.0 ± 1.3	3033.9 ± 180.8
	0	99.6 ± 12.6	92.3 ± 3.2	112.9 ± 6.4	20.2 ± 3.3†	2286.3 ± 104.5
	4	117.9 ± 15.5	90.8 ± 5.2	112.2 ± 7.3	25.0 ± 3.6	2246.1 ± 123.3
	8	107.9 ± 15.1	93.0 ± 5.3	112.4 ± 8.3	23.6 ± 7.4	2311.2 ± 202.9

Pre-CHF: prior to microembolization-induced myocardial infarction. Time 0 is after induction of CHF and before ASM or SAL injection. MAP, mean arterial pressure; HR, heart rate; LVSP and LVEDP, left ventricular systolic and end diastolic pressure; LV dP/dt_{max}, maximal change in LV systolic pressure per second.
*$p < 0.05$ ASM compared to SAL group.
†$p < 0.05$ compared to pre-CHF level.

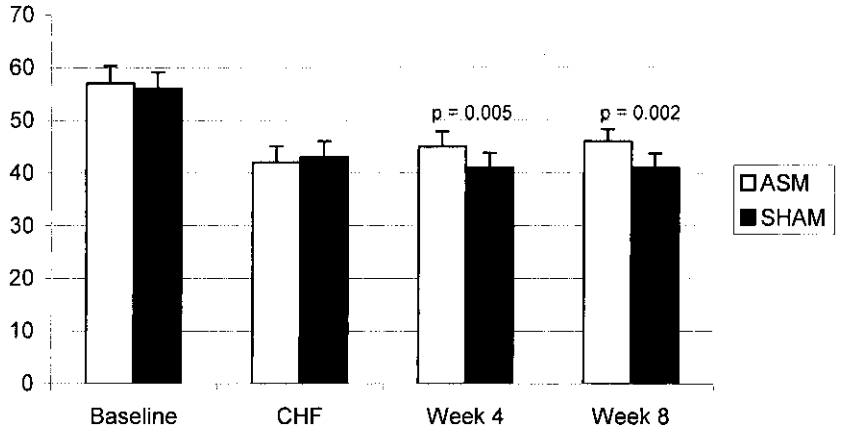

Figure 2. Serial echocardiography, demonstrating a significant increase in ejection fraction in the ASM group at 4 and 8 weeks after injection.

PVCs at week 4 sustained an unobserved death 1 week later. The cause of death in the former dog was not evident on gross and histopathologic inspection.

Ventricular tachycardia was monomorphic in all instances, with maximal QRS duration 180 ms, and most often with rates between 120 and 160 bpm. Brief episodes of rapid VT (>200 bpm) were observed infrequently. VT was generally nonsustained (10–20 s). Sustained VT was seen in two animals, one from each treatment group.

In the entire group, 27% developed ventricular tachycardia on AECGs following study intervention. Three of nine (33%) were in the ASM group and one of six (17%) were in the SAL group (primary end-point, p = ns, Fisher exact test) (Fig. 3). The same animal had VT at both 4 and 8 weeks in the SAL group. After incorporating the two periprocedure deaths (one from each group) and the unobserved deaths (one in the ASM group), 5 of 10 dogs (50%) in the ASM group and two of seven (29%) SAL-injected animals developed ventricular tachycardia after injection, also not significant ($p = 0.87$).

Supraventricular arrhythmias were not seen in either group. Episodes of 3–4-s pauses occurred in one animal in the ASM group that had also developed VT.

Heart Rate Variability

Table 4 contains values for HR and several time–domain parameters of HRV. Baseline values for all parameters were similar in both groups and not different in follow up in either ASM or SAL group, although a trend to higher SDNNRR was noted in the SAL group ($p = 0.08$). However, there was a significant reduction in SDNNRR at 8 weeks following the administration of ASM when compared to saline (174 ± 78 vs. 276 ± 78 s, $p = 0.03$). No differences were observed in RMSSD or pNN50.

Table 3. Ambulatory Arrhythmias

			PVCs			VT		
	Time (Weeks)	*N* Tot	Per Hour (Range)	*N* With >30/h	*N* With VT	No. Events	$Rate_{max}$ (bpm)	$Duration_{max}$ (min)
ASM	0	11	0	0	0	—	—	—
	4	9	420 (0–2907)	1	1	2	234	0.01
	8–10	8	4 (0–85)	2	2	248	146	0.80
SAL	0	8	8 (0–35)	3	1	113	177	0.30
	4	6	18 (0–104)	1	1	2	234	0.10
	8–10	5	4 (0–74)	2	1	328	187	0.64

Frequency of ventricular arrhythmias (PVC, premature ventricular contractions; VT, ventricular tachycardia) at three observation times, from ambulatory ECG (AECG) recordings. Times are as in Table 2. *N* Tot, total number in the group with evaluable AECGs; PVC, premature ventricular contractions; VT, ventricular tachycardia; $Rate_{max}$, maximal VT rate in bpm; $Duration_{max}$, maximal VT duration in minutes.

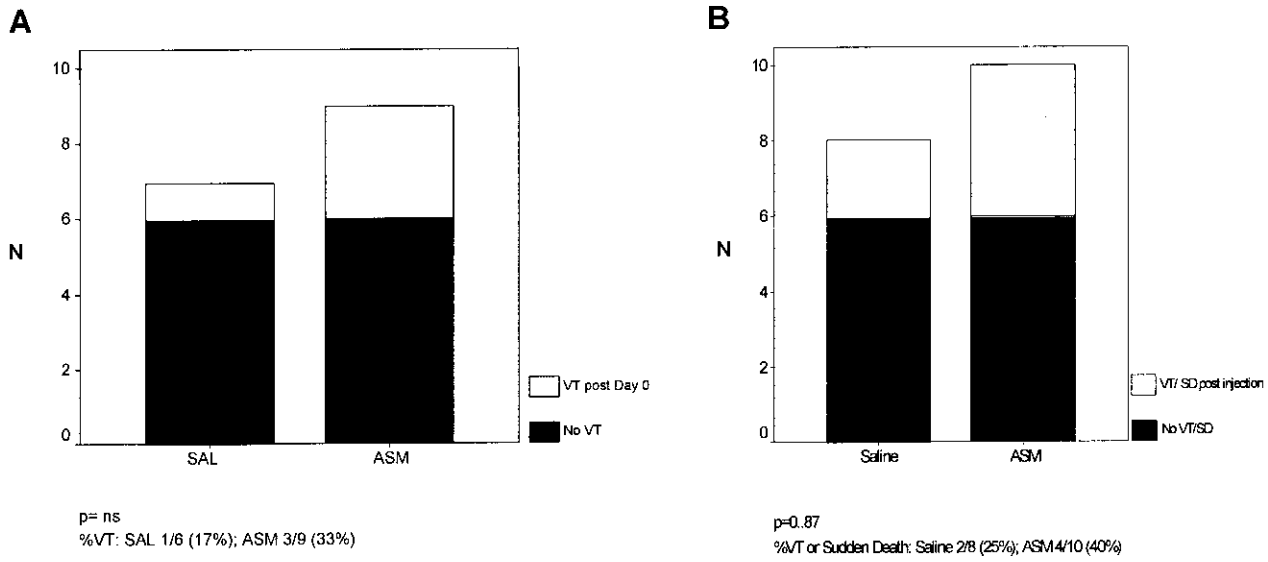

Figure 3. Incidence of ventricular tachycardia (VT) at any time after assignment to ASM or SAL, compared to pretreatment CHF baseline: SAL one of six (17%); ASM three of nine (33%). There was no intergroup difference in the occurrence of VT following treatment assignment (Fisher exact test).

DISCUSSION

The possibility of restoring systolic function to post-MI ventricles through the repletion of contractile cells presents an exciting opportunity for patients with CHF. One approach to accomplish this is by delivering immature myogenic cells, such as ASM, directly into areas of myocardial fibrosis (2), anticipating that differentiation, engraftment, and integration with host cardiomyocytes will enhance regional kinetics. In order for myogenic cells to fully integrate with host tissue, and to therefore optimize their effects, gap junctions must be forged. ASM, despite their capacity for connexin-43 production (9), do not form gap junctions (18,26,37) and, therefore, remain electrically insulated from host myocardium. In this state, ASM grafts may create an environment favorable to reentry and the initiation ventricular tachyarrhythmias, a risk suggested by early clinical observations (20). In light of these findings and the paucity of arrhythmia-directed preclinical studies, further characterization of the relationship of ASM implantation and ventricular tachyarrhythmias is warranted.

The objective of the present study was to compare the frequency of arrhythmias following intramyocardial injection of either ASM or SAL (study intervention) in the chronic post-MI ventricle. Microsphere coronary

Table 4. Heart Rate Variability

	Time	*N*	HR (bpm)	Mean RR (ms)	SDNN RR (ms)	RMSSD	pNN50 (%)
ASM	0	11	98 ± 9	640 ± 62	217 ± 62*	229 ± 50	52 ± 10
	4	10	105 ± 23	610 ± 127	174 ± 95	188 ± 121	42 ± 26
	8–10	9	103 ± 19	622 ± 101	174 ± 78*‡	212 ± 109	46 ± 20
SAL	0	8	100 ± 12	624 ± 91	210 ± 60†	268 ± 70	45 ± 25
	4	6	101 ± 6	645 ± 37	242 ± 19	301 ± 33	64 ± 5
	8–10	5	102 ± 17	684 ± 117	276 ± 78†‡	362 ± 138	59 ± 15

Measures of heart rate variability. SDNNRR, standard deviation of normal-to-normal RR intervals; RMSSD, root mean square of successive NN differences; pNN50, percentage of adjacent NN intervals in a 24-h recording, which differ by at least 50 ms.
*ASM 0 week versus 8 weeks, p = ns.
†SAL 0 week versus 8 weeks, $p = 0.08$.
‡ASM versus SAL week 8, $p = 0.03$.
All other intra- and intergroup comparisons, p = ns.

embolization resulted in extensive left ventricular dysfunction and CHF, demonstrated by increases in LVEDP and reductions in LV dP/dt_{max}, simulates the human condition of post-MI CHF and, in doing so, serves as an effective model in which to study ventricular arrhythmias (29). The recovery of LV function (12) afforded by ASM in this study provides a well-developed model for the study of adverse consequences of cell therapy in CHF. An arrhythmia surveillance protocol was constructed in which AECG recordings were sampled at specified times up to 10 weeks following study intervention.

The intramyocardial delivery of ASM, by either surgical and catheter-based approaches in doses of 270 to 789×10^6 cells, is similar to methods employed in clinical studies (13,20,23,34). Unlike some studies, cell injections were not constrained only to the infarct zone, but were extended beyond its margins, therein maximizing interactions between implants and recipient tissue, be they beneficial or adverse. The positive effects of cell injection were significant. Compared to animals receiving SAL, LVEDP remained lower and LV dP/dt_{max} higher over the 8 weeks after ASM implantation. Moreover, a significant, progressive increase in echocardiographic ejection fraction was seen in the ASM group. Thus, while assessment of the functional consequences of ASM was not the primary purpose of this study, these observations strongly indicate that the effects of cell implantation, however mediated, were present throughout the duration of the study.

The predominant ambulatory arrhythmia observed during the course of this study was ventricular, with 37% demonstrating either frequent PVCs (>30/h) or ventricular tachycardia. This is comparable to that reported in a similar microembolism model of canine heart failure (29), in which 33% of animals developed VT on AECG over the 16 weeks following the induction of heart failure. In our study, ventricular tachycardias were clustered on baseline AECGs in the SAL group, whereas in the ASM group VT occurred only in the postinjection evaluation period. Compared to the SAL group, the likelihood of developing VT following ASM implantation was not significantly different, although there was a trend toward a higher frequency. This was also the case when the three postintervention sudden deaths were incorporated into the VT statistic. Therefore, enhanced arrhythmogenicity post-ASM implantation, either by VT (primary endpoint) or PVC frequency, was not demonstrable by the assessment tool used in this study. Additionally, the characteristics of VT, including morphology and rate, did not appear to differ between the two groups. The incidences of supraventricular tachyarrhythmias or bradyarrhythmias were very small.

There are few reports of heart rate variability (HRV) in dogs, most describing findings in normal animals without cardiac disease (22). Our findings demonstrate a reduction in SDNNRR in dogs following the administration of ASM in a CHF model, without a clear relationship to VT. The lack of an association between the two may be due to the low frequency of arrhythmias in the total population.

Clinical reports link HRV to a poorer prognosis in patients with either heart failure or recent infarction (8,10,16). In a prospective study of amiodarone therapy in patients with CHF (ischemic or nonischemic), Bilchick et al. (4) described a strong association of overall mortality and sudden death among those in the lowest quartile of SDNNRR (<65.3 ms) and proposed that an improvement in both outcomes are linearly associated with 10-ms increments in this particular HRV metric. The relationship of HRV to ventricular arrhythmias is not clear (25).

There are several limitations to our study, from standpoints of design and clinical extrapolation. First, the infarct model in our study leads to patchy fibrosis, rather than the diffuse pattern arising from epicardial coronary artery occlusion. And the use of both surgical (epicardial) and catheter (endocardial) injection techniques further divides the two treatment groups. While the comparative risk of arrhythmias, either at baseline or following cell implantation, in these pathologic conditions is not known, one might expect a greater incidence in our model, which may provide a fertile substrate for reentrant mechanisms. Regardless of these differences, the inclusion of saline control groups mitigates bias introduced by both the disease model and injection strategy.

Second, AECGs sampling and analysis, while frequent in comparison to published clinical series (13, 20,35), may have been occurred too late to adequately reflect the true incidence of ventricular arrhythmias, which is believed to peak at 10 to 22 days (20) after cell implantation. The incidence reported here is therefore likely a minimal estimate. Nevertheless, in that fatal arrhythmias did not occur between days 2 and 35, the clinical relevance of our findings may still apply. Additionally, we did not attempt to identify mechanisms of arrhythmia genesis in this study, which would have required ventricular stimulation studies or signal average ECGs, or both.

Third, animals were not treated with either beta blockers or angiotensin converting enzyme inhibitors, but rather received only diuretics as indicated for fluid management. As above, withholding such agents, especially beta blockers, would theoretically elevate the risk of ventricular arrhythmias in our population, equally affecting all treatment groups. Also, the administration of

antiarrhythmic agents as primary prophylaxis was not entertained in this observational study (and is of uncertain value in patients), and their use in secondary prophylaxis was outside the logistical scope of AECG interpretation.

Importantly, a study the size of ours is susceptible a type 2 error. The frequency of daily ventricular arrhythmias was wide, with a few animals displaying large numbers of PVCs or runs of VT, while other animals had none. A study with the primary objective of detecting differences in postimplantation VT would require an $n = 400$ for a power of 0.8 to detect a value of $p = 0.05$. Moreover, seven animals did not complete the study. In addition to the five deaths noted above, two others had AECG data that were insufficient to be included in the arrhythmia analysis.

The safety of cellular cardiomyoplasty with ASM has been challenged by recent reports of ventricular tacharrhythmias, resulting in the incorporation of antiarrhythmia therapy (including implantable cardioverter defibrillators) into several reported (19,33) and ongoing (SEISMIC, MYOHEART) clinical studies. In order for causal links to be made between ASM implantation and ventricular arrhythmias, investigators will need both to identify proarrhythmic interactions between implanted and recipient cells and to conduct controlled clinical trials that are adequately designed to detect arrhythmic events. While the latter may be burdensome for study participants, due to the need for frequent arrhythmia monitoring, the knowledge gained will be invaluable in defining the role of cellular cardiomyoplasty in the clinical setting.

To our knowledge, this is the first study in large animals to report the effects of ASM implantation on the occurrence of ventricular arrhythmias. In these data we have found no association between the two. The finding of reduced HRV after ASM implantation warrants further study, especially in the clinical setting.

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