

SUPPLEMENTAL MATERIAL

METHODS

Induction of myocardial infarction and tissue harvest

Male Yorkshire pigs (13.5 ± 0.8 kg, age 8–10 weeks) were anesthetized with an i.m. injection of ketamine (33 mg/kg) followed by an i.v. bolus of sodium pentobarbital (30 mg/kg). The animals were intubated and ventilated with 100% O₂; anesthesia was maintained with isoflurane (0.8-1.5%). Under sterile conditions, the heart was exposed by a median sternotomy and suspended in a pericardial cradle. The right atrial appendage was cross-clamped and the tip (1-2 g) resected for isolation of CSCs; the atrium was then sewed with a 5.0 prolene suture. The harvested atrial samples were rinsed in PBS, cut into small (1-2 mg) pieces, and snap frozen in a freezing medium composed of the growth culture medium pre-mixed with DMSO (9:1 vol/vol). The growth medium consisted of Ham's F12 (BioWhittaker), 10% fetal bovine serum (Gibco) and penicillin/streptomycin (BioWhittaker).

A snare (3-0 silk suture) was placed around the left anterior descending (LAD) coronary artery, distal to the origin of the second diagonal branch. To produce MI, the snare was tightened for 90 min and then released; coronary reperfusion was visually confirmed by the reactive hyperemia. Pigs received lidocaine (2 mg/kg i.v. bolus followed by a 0.05 mg/kg/min infusion) and amiodarone (2 mg/kg i.v. bolus followed by a 0.04 mg/kg/min infusion) starting before coronary occlusion and ending 30 min after reperfusion. The chest was closed in layers. Animals received postoperative antibiotics (gentamicin 5 mg/kg i.m. and cefazolin 30 mg/kg i.m. daily for 3 d) and ketoprofen (2.5 mg/kg i.m. every 24 h for the first 48 h). A group of non-operated pigs was studied as normal controls. These animals did not undergo any surgical procedure and were

monitored for a period of time equivalent to the infarcted animals.

Cardiac enzyme measurement

Blood samples were obtained for serial measurement of cardiac markers before and immediately after catheterization and at 6, 12, 24 and 48 h thereafter. Cardiac markers, which included creatinine kinase (CK), creatinine kinase-MB fraction (CK-MB), troponin I (TnI), troponin T (TnT), myoglobin, and lactate dehydrogenase (LDH), were measured using standard human kits. As the cutoffs of normalcy for these proteins in pigs are unknown, relative changes in blood levels were utilized for intergroup comparisons (all of the aforementioned human assays were used after obtaining evidence of cross reactivity with porcine enzymes).

Echocardiographic and hemodynamic studies

Echocardiograms were obtained at baseline (before CSC delivery) and 31 d after CSC delivery (just before sacrifice) using an HDI 5000 ultrasound system (Philips Medical Systems) equipped with 4-2 MHz and 7-4 MHz phased array transducers. Before the echocardiographic study, pigs were anesthetized (isoflurane) and placed in the left lateral decubitus position. Temperature was monitored with a rectal temperature probe and kept between 37.0°C and 37.5°C with a heating pad. The parasternal long-axis, parasternal short-axis, and apical four-chamber views were used to obtain 2D, M-mode, and spectral Doppler images ^{1, 2}. Systolic and diastolic anatomic parameters were obtained from M-mode tracings at the mid-papillary level. Digital images were analyzed off-line by a single blinded observer using ProSolv (version 2.5) image analysis software (Problem Solving Concepts. Inc., Indianapolis, IN) according to the American Society of Echocardiography standards ³.

Two sets of hemodynamic measurements were performed under general anesthesia, before the start of the catheterization procedure and 31 d later (just before sacrifice). The left carotid artery was instrumented with a 6F sheath through which a 6F Millar micro-tip catheter pressure transducer (Millar Instruments Inc., Houston, TX) was placed and advanced into the LV cavity.

REFERENCES

1. Dawn B, Stein AB, Urbanek K, Rota M, Whang B, Rastaldo R, Torella D, Tang XL, Rezazadeh A, Kajstura J, Leri A, Hunt G, Varma J, Prabhu SD, Anversa P, Bolli R. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci U S A*. 2005;102:3766-3771
2. Stein AB, Tiwari S, Thomas P, Hunt G, Levent C, Stoddard MF, Tang XL, Bolli R, Dawn B. Effects of anesthesia on echocardiographic assessment of left ventricular structure and function in rats. *Basic Res Cardiol*. 2007;102:28-41
3. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American society of echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. *J Am Soc Echocardiogr*. 1989;2:358-367

Supplemental table. Physiologic variables

		Vehicle-treated (n=10)	CSC-treated (n=11)
Body weight (kg)	At Surgery	13.1 ± 1.0	14.0 ± 0.7
	At Catheterization	43.4 ± 5.7	41.8 ± 3.3
	Week 1	46.0 ± 6.2	44.2 ± 3.6
	Week 2	48.6 ± 6.2	46.7 ± 3.7
	Week 3	50.6 ± 6.6	49.1 ± 3.5
	Week 4	52.6 ± 6.6	51.7 ± 3.4
Heart rate (bpm) during surgery	Baseline (pre-thoracotomy)	116 ± 5	118 ± 12
	Pre-occlusion	109 ± 5	106 ± 10
	30 min occlusion	96 ± 6	91 ± 6
	60 min occlusion	94 ± 4	87 ± 5
	90 min occlusion	95 ± 7	85 ± 5
	15 min reperfusion	92 ± 5	85 ± 6
	30 min reperfusion	90 ± 5	84 ± 6
	60 min reperfusion	81 ± 3	80 ± 5
Mean arterial pressure (mmHg) during surgery	Baseline (pre-thoracotomy)	67 ± 5	75 ± 7
	Pre-occlusion	72 ± 4	70 ± 8
	30 min occlusion	57 ± 4	65 ± 5
	60 min occlusion	58 ± 3	61 ± 5
	90 min occlusion	59 ± 3	60 ± 3
	15 min reperfusion	56 ± 2	57 ± 3
	30 min reperfusion	55 ± 2	58 ± 3
	60 min reperfusion	56 ± 2	55 ± 3
Electrolytes and hemoglobin before catheterization	Hemoglobin	9.2 ± 0.4	9.9 ± 0.3
	K ⁺ (mmol/L)	4.0 ± 0.2	4.0 ± 0.1
	Na ⁺ (mmol/L)	132.1 ± 3.6	136.8 ± 2.2
	HCT (%)	27.6 ± 0.7	27.4 ± 1.3
Electrolytes and hemoglobin after catheterization	Hemoglobin	11.6 ± 0.7*	13.1 ± 1.2*
	K ⁺ (mmol/L)	4.6 ± 0.2	4.4 ± 0.2
	Na ⁺ (mmol/L)	129.0 ± 3.0	132.3 ± 3.6
	HCT (%)	30.5 ± 3.0	25.4 ± 1.4
Heart rate (bpm) during catheterization	Pre inflation I	77 ± 2	89 ± 6
	During inflation I	77 ± 2	84 ± 4
	Pre-inflation II	77 ± 2	84 ± 4
	During inflation II	76 ± 2	82 ± 4
	Pre-inflation III	78 ± 2	88 ± 5
	During inflation III	76 ± 2	86 ± 4
Mean arterial pressure (mmHg) during catheterization	Pre inflation I	79 ± 5	68 ± 3
	During inflation I	72 ± 8	62 ± 2
	Pre-inflation II	75 ± 6	61 ± 2
	During inflation II	72 ± 8	63 ± 2
	Pre-inflation III	67 ± 7	65 ± 3
	During inflation III	67 ± 7	60 ± 2

* $P < 0.05$ vs. value before catheterization.