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Intravenous infusion of cardiac progenitor cells in animal models of single ventricular physiology

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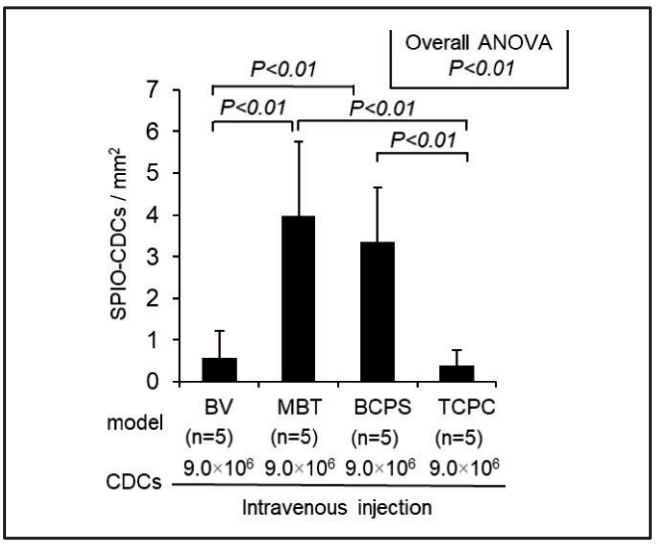
Intravenously injected CDCs were detected in the myocardium of pig MBT and BCPS models but not in TCPC model.

Summary

Three-stage right heart bypass model was established in pig to study early cardiac homing of the intravenously transplanted CDCs. Cardiac retention of delivered CDCs was significantly higher in the MBT and BCPS groups than in the TCPC group. Intravenous CDCs delivery through the lung bypass circuit is effective in targeting a single ventricle before TCPC completion.



Legend: BCPS: bidirectional cavopulmonary shunt; BV: biventricular; CDCs: cardiosphere-derived cells; MBT: modified Blalock–Taussig shunt; SPIO: superparamagnetic iron oxide; TCPC: total cavopulmonary connection

Abstract

OBJECTIVES: The goal of this study was to identify the practical applications of intravenous cell therapy for single-ventricle physiology (SVP) by establishing experimental SVP models.

METHODS: An SVP with a three-stage palliation was constructed in an acute swine model without cardiopulmonary bypass. A modified Blalock–Taussig (MBT) shunt was created using an aortopulmonary shunt with the superior and inferior venae cavae (SVC and IVC, respectively) connected to the left atrium ($n = 10$). A bidirectional cavopulmonary shunt (BCPS) was constructed using a graft between the IVC and the left atrium with an SVC cavopulmonary connection ($n = 10$). The SVC and the IVC were connected to the pulmonary artery to establish a total cavopulmonary connection

(TCPC, $n = 10$). The survival times of half of the animal models were studied. The other half and the biventricular sham control (n = 5) were injected intravenously with cardiosphere-derived cells (CDCs), and the cardiac retention of CDCs was assessed after 2 h.

RESULTS: All SVP models died within 20 h. Perioperative mortality was higher in the BCPS group because of lower oxygen saturation ($P < 0.001$). Cardiac retention of intravenously delivered CDCs, as detected by magnetic resonance imaging and histologic analysis, was significantly higher in the modified Blalock-Taussig and BCPS groups than in the TCPC group ($P < 0.01$).

CONCLUSIONS: Without the total right heart exclusion, stage-specific SVP models can be functionally constructed in pigs with stable outcomes. Intravenous CDC injections may be applicable in patients with SVP before TCPC completion, given that the initial lung trafficking is efficiently bypassed and sufficient systemic blood flow is supplied from the single ventricle.

Keywords: animal model • single ventricular physiology • cell therapy • cardiosphere-derived cell • intravenous

ABBREVIATIONS AND ACRONYMS

BCPS	bidirectional cavopulmonary shunt
BV	biventricular
CDC(s)	cardiosphere-derived cell(s)
ePTFE	expanded polytetrafluoroethylene
IVC	inferior vena cava
LA	left atrium
LV	left ventricle
MBT	modified Blalock–Taussig
MRI	magnetic resonance imaging
PA	pulmonary artery
SPIO	superparamagnetic iron oxide
SVC	superior vena cava
SVP	single-ventricle physiology
TCPC	total cavopulmonary connection

INTRODUCTION

Stem cell therapies have recently been shown to be feasible and safe interventions for treating patients with congenital heart diseases, such as dilated cardiomyopathy and single-ventricle lesions [1, 2]. Nevertheless, a variety of beneficial effects corresponding to the results of clinical trials have not yet been fully elucidated in appropriate experimental models that precisely reflect in vivo human pathophysiological conditions.

Experimental studies that recapitulate single-ventricle physiology (SVP) are extremely challenging and technically difficult. No researchers have successfully achieved single-ventricle circulation in small animals, and a large chronic animal model has not been established even for the use of ventricular assist devices in the postnatal heart [3]. Therefore, our goal was to establish novel animal models of single-ventricle circulation in pigs to assess surgical palliation-specific haemodynamics during the creation of single-ventricle circulation, allowing the development of a new cell transplant strategy and outcome evaluation of stem cell distribution with respect to the route of delivery.

MATERIAL AND METHODS

Ethics statement

The experimental protocol was approved by the Experimental Animal Committee of the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (approval number: OKU-2018941; approval date: 27 March 2019). All experimental animals were cared for in accordance with the institutional guidelines and the Guide for the Care and Use of Laboratory Animals [4].

Study design and end point

Three-stage palliation models of the SVP were established to mimic circulation with univentricular disease. These models included a modified Blalock–Taussig shunt, a bidirectional cavopulmonary shunt (BCPS) and a total cavopulmonary shunt model.

First, the haemodynamic features of 5 piglets from each model and control group were compared ($n = 5 \times 4$), and the SVP models were observed from extubation until death.

Next, the piglets with biventricular (BV) circulation were compared using different methods of stem cell administration, and each ventricular model received an intravenous stem cell injection. This study included 35 pigs: a control group of 5 pigs, and 2 sets of 3 treatment groups, each including 5 pigs that shared a common control. Ten piglets with BV circulation received intracoronary injections of a medium ($n = 5$) and stem cells ($n = 5$). Five piglets received an intra-left ventricular injection of stem cells ($n = 5$). Five piglets of each SVP model and the common control group (BV circulation) received intravenous injections of stem cells ($n = 5 \times 4$). These 7 groups were observed for 2 h under general anaesthesia with artificial ventilation and then were sacrificed to extract their hearts. The hearts were examined using ex vivo cardiac magnetic resonance imaging (MRI). Finally, the hearts of the 3 SVP model groups and the control group with intravenous injection were analysed histologically.

The end point of the observation of the survival time was death.

The primary end point of this study was stem cell retention in the cardiac muscle after intravenous injection.

Preparation of animal models

Eight-week-old Yorkshire pigs weighing 25 kg were anaesthetized via inhalation of 2% isoflurane. After performing a median sternotomy and administering 4000 units of heparin, we performed the appropriate surgical procedure. For comparison, normal Yorkshire pigs with BV hearts underwent a median sternotomy as a control.

To assess postoperative survival, the sternum was closed, and the animal was extubated when adequate spontaneous respiration occurred.

Modified Blalock–Taussig shunt in a swine single-ventricle physiology model as a stage 1 palliation model

After a median sternotomy, the proximal part of the brachiocephalic artery was clamped and a 6-mm diameter expanded polytetrafluoroethylene (ePTFE) tube was anastomosed in a side-to-end

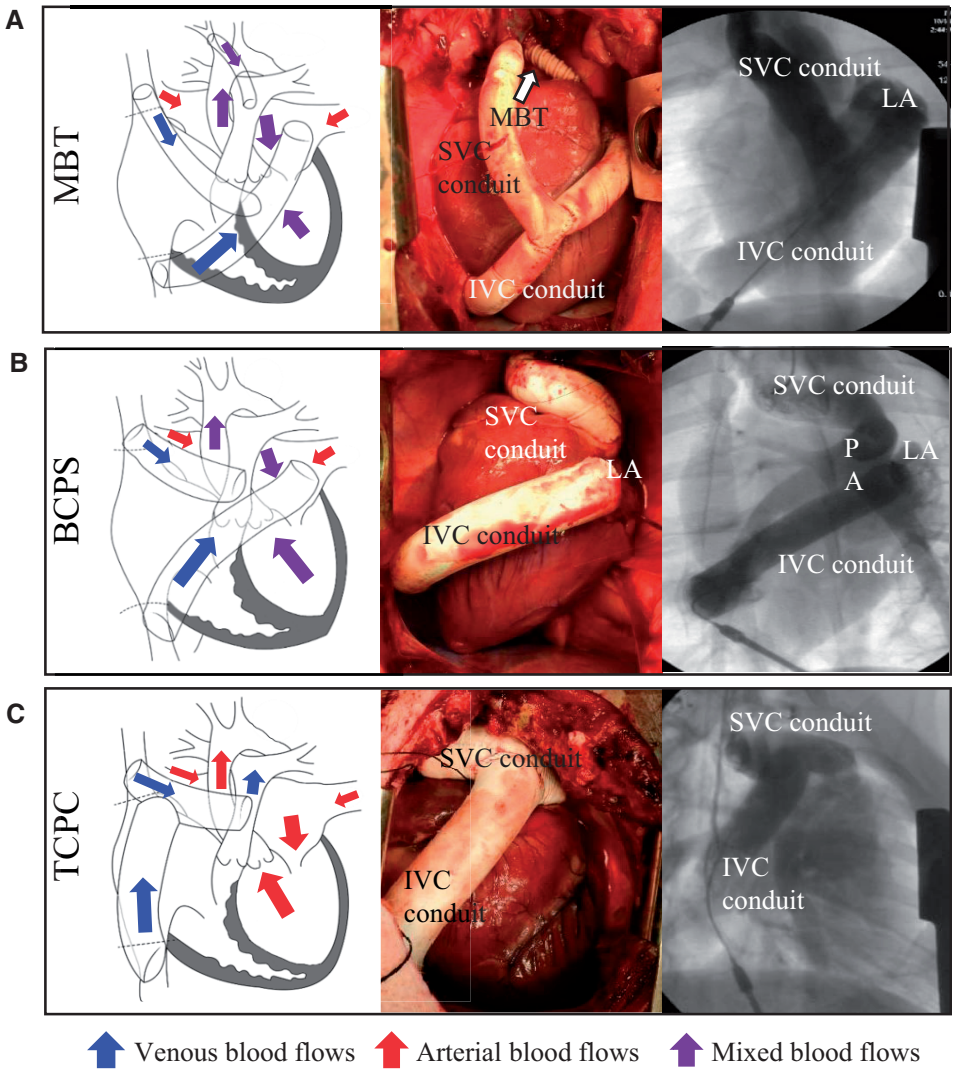


Figure 1: Stage-specific swine models of single-ventricle physiology. The experimental models of single-ventricular circulation with a modified Blalock–Taussig shunt (**A**), a bidirectional cavopulmonary shunt (**B**) and a total cavopulmonary connection (**C**) and their angiograms are shown. BCPS: bidirectional cavopulmonary shunt; IVC: inferior vena cava; LA: left atrium; MBT: modified Blalock–Taussig; PA: pulmonary artery; SVC: superior vena cava; TCPC: total cavopulmonary connection.

fashion using a continuous 6–0 polypropylene suture (Prolene, Ethicon, Raritan, NJ, USA). The distal end of the conduit was anastomosed to the main pulmonary artery (PA) in an end-to-side fashion under partial clamping of the PA, creating a direct systemic-to-pulmonary shunt (modified Blalock–Taussig shunt; Fig. 1A).

Venous blood flowing from the superior and inferior venae cavae (SVC and IVC, respectively) was drained into the left atrium (LA) by direct grafting of prosthetic conduits in a Y-configuration with a 14-mm diameter ePTFE. Single-ventricular circulation was established by ligating the SVC and IVC at the atrial junction (Fig. 1A).

Bidirectional cavopulmonary shunt in a swine single-ventricle physiology model as a stage 2 palliation model

A bidirectional cavopulmonary shunt (BCPS) was constructed by connecting the IVC to the LA and the SVC to the main PA with 2 prosthetic conduits (Fig. 1B). After the IVC-to-LA anastomosis was performed as described previously, the SVC and PA were connected to the ePTFE graft under partial clamping of the PA with azygos vein ligation. When all the anastomoses were completed, both the SVC and IVC were ligated at the right atrial junction.

Total cavopulmonary connection in a swine single-ventricle physiology model as a stage 3 palliation model

A prosthetic conduit with 2 branches was constructed to deliver total venous return directly into the PA as a total cavopulmonary connection (TCPC). This procedure was completed by graft anastomosis of the SVC to the PA, and another graft was anastomosed between the IVC and the graft in a T-configuration (Fig. 1C).

Haemodynamic measurements and angiography

Haemodynamic measurements in the swine models were performed during intubation when the haemodynamics stabilized after model generation.

Haemodynamic variables in pigs were measured directly by placing a 22-gauge needle connected to a pressure transducer (TruWave disposable pressure transducer; Edwards Lifesciences, Irvine, CA, USA) into the location of interest. The total cardiac output and systemic blood flow were measured using a pressure–volume system (ADV500 PV System, Transonic System, NY, USA). Vascular resistance was calculated using the following formula: Systemic ventricular resistance = (mean arterial pressure–mean IVC pressure)/cardiac output*79.92 (dyne*sec/cm^5). In this formula, the IVC pressure was adopted for the precardiac pressure instead of the right atrial pressure. Pulmonary vascular resistance was calculated as (mean pulmonary artery pressure–mean LA pressure)/cardiac output*79.92 (dyne*sec/cm^5). Selective angiography was performed to check the postoperative blood flow.

Preparation of cardiosphere-derived cells

Piglet CDCs were prepared as described by Sano *et al.* [5].

Piglet right atrial tissues were minced and treated with 0.4% type II collagenase and 0.01% DNase I. Small atrial tissues were cultured to grow cardiospheres in Dulbecco's modified Eagle medium and F12 medium supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin.

CDCs were labelled with superparamagnetic iron oxide (SPIO) (Dragon Green, Bangs Laboratories, Fishers, IN, USA). CDCs were cultured in SPIO-supplemented medium (30 μL Dragon Green/10 ml medium) overnight.

Cell therapy

The CDCs were prepared for transplant and verified as previously described [5, 6].

Cell therapy was performed using the following 3 techniques: (i) Intracoronary CDC infusion was performed during temporary occlusion of the aorta, SVC and IVC via the aortic route. Both the SVC and IVC were then declamped to wash out the CDCs into the coronary artery. After achieving steady-state cardiac contraction, the aorta was declamped. (ii) Intraventricular infusion of CDCs was performed directly into the left ventricle (LV) without aortic clamping. (iii) Intravenous delivery of the CDCs was performed by direct injection from the IVC.

The dose of CDCs administered to the swine LV and each model was 300,000 cells/kg of body weight, which was equivalent to that used in previous studies [5]. Preliminary results in rats showed that similar delivery to the myocardium was expected when 10 times the number of CDCs for coronary injection was administered into the LV. Therefore, the intracoronary administration was 30,000 cells/kg.

Magnetic resonance imaging

Ex vivo MRI experiments in pigs were performed using a 1.5-Tesla Signa EXCITE MRI Scanner System with an 8-channel head array coil (GE Healthcare, Chicago, IL, USA) to assess cell distribution ex vivo. Two hours after administering CDCs with an artificial respirator and with their chest left open, the pigs' hearts were excised and transferred to the MRI suite; T2* weighted images of the extracted heart were acquired [6]. The following settings were applied for T2* weighted imaging: repetition time, 500 ms; echo time, 4.5 ms; field of view, 24 × 24 cm; slice thickness, 5 mm; gap, 0 mm; data matrix, 512 × 512; and flip angle, 30°.

Semi-quantitative analysis of superparamagnetic iron oxide-labelled cells in magnetic resonance imaging

The MRI data were analysed using ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA). The Digital Imaging and Comunications in Medicine images were converted to tagged image file format to analyse the data using the ImageJ software. To calculate the ratio of the low-signal-intensity area to the total organ area, a low-intensity area was selected and measured semi-automatically. The low-intensity area was measured in a representative slice of each organ, and the ratio to the whole area of the organ was calculated.

Histologic analysis

Myocardial sections were deparaffinized, dehydrated, stained with a Prussian Blue Iron Stain Kit (Polysciences, Warrington, PA, USA), and counterstained with haematoxylin and eosin to detect intracellular SPIO distribution in the transplanted myocardium. The morphology and structure of the in vivo–engrafted CDCs were observed in stacked images using a BZ-X700 Analyzer (Keyence, Osaka, Japan). The number of Prussian blue-positive cells was calculated and corrected with those from the total cardiac tissues analysed.

Statistical analyses

Data are expressed as mean ± SD. Normal distribution was tested using the Shapiro–Wilk test. Multiple comparisons of MRI, histologic analysis data and haemodynamic parameters among the study groups were performed using one-way analysis of variance, followed by Tukey's post-hoc correction that adjusted for multiplicity in comparing multiple same-size groups that had a normal distribution and standard deviation. Survival after a specified cardiac procedure was compared using log-rank tests to assess the distribution of time to death, which may differ among the surgical procedures. Statistical analyses were conducted using the SPSS software (version 27, IBM, Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

Desirable sample size was calculated with G*Power3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html,Heinrich-Heine-Universität, Düsseldorf).

RESULTS

Stage-specific swine models of single-ventricle physiology

To study CDC homing and engraftment in large animals, we developed 3 distinct palliative stage surgical models with single-

Table 1: Haemodynamic measurements in swine models with a single ventricle

	BV (n = 5)	MBT (n = 5)	BCPS (n = 5)	TCPC (n = 5)	*P*
HR (bpm)	81.2 ± 8.2	83.8 ± 5.8	73.0 ± 3.1	77.4 ± 9.3	0.13
Mean ABP (mmHg)	65.4 ± 2.7	50 ± 6.7*	54.7 ± 5.0	62.4 ± 5.2	<0.01
Systolic ABP (mmHg)	85.6 ± 7.3	73.4 ± 13.6	77.8 ± 7.5	81.8 ± 5.9	0.21
Diastolic ABP (mmHg)	55.8 ± 1.8	38.4 ± 3.8*	43.2 ± 4.0*	52.8 ± 6.4	<0.01
Mean PAP (mmHg)	13.2 ± 0.5	21.8 ± 2.4	12.2 ± 1.3	14.8 ± 0.8	<0.01
LAP (mmHg)	5.4 ± 1.5	7.6 ± 1.1	8.6 ± 0.5	4.6 ± 1.1	<0.01
SVCP (mmHg)	4.4 ± 0.5	4.2 ± 1.6	12.2 ± 1.3†	13.8 ± 0.4[a]	<0.01
IVCP (mmHg)	4.8 ± 0.8	5.2 ± 0.8	5.0 ± 1.6	14.8 ± 0.8[b]	<0.01
SVR(dyne*sec*cm^{-5})	1851.7 ± 264.4	1913.4 ± 457.8	1760.4 ± 180.2	2127.1 ± 199.2	<0.01
PVR(dyne*sec*cm^{-5})	236.7 ± 42.1	345.3 ± 33.4	458.8 ± 153.0	456.4 ± 65.7	<0.01
CO (L/min)	2.7 ± 0.27	4.4 ± 0.14	2.3 ± 0.15	1.8 ± 0 0.1	<0.01
SV (mL)	31.4 ± 4.5	53.2 ± 2.5	31.0 ± 0.77	23.4 ± 3.2	<0.01
EDV (mL)	67.9 ± 5.8	111.1 ± 4.5	58.8 ± 2.8	46.2 ± 4.4	<0.01
ESV (mL)	36.5 ± 4.0	57.9 ± 6.6	27.8 ± 2.4	23.0 ± 1.4	<0.01
EF (%)	46.3 ± 4.5	48.0 ± 3.8	52.7 ± 1.9	50.3 ± 2.6	<0.01
SaO_2 (%)	99 ± 0.8	80.4 ± 6.5[c]	48.8 ± 4.6[d]	99.2 ± 0.8	<0.01
$SpaO_2$ (%)	68.2 ± 6.0	75 ± 4.6[c]	32.4 ± 3.5[d]	61.6 ± 5.3	<0.01
Op time (min)	NA	123.6 ± 10.9[e]	94.6 ± 13.4	92 ± 12.1	<0.01
Survival (h)	NA	9.4 ± 5.0	1.8 ± 0.8**	9.8 ± 5.6	0.02

Data are expressed as mean ± SD.

*$P < 0.05$ vs BV and TCPC groups.

** $P < 0.05$ vs MBT and TCPC groups.

[a]$P < 0.01$ vs BV and MBT groups.

[b]$P < 0.01$ vs BV, MBT, and BCPS groups.

[c]$P < 0.05$ vs BV, BCPS, and TCPC groups.

[d]$P < 0.01$ vs BV, MBT, and TCPC groups.

[e]$P < 0.05$ vs BCPS and TCPC groups.

ABP: arterial blood pressure (mmHg); BCPS: bidirectional cavopulmonary shunt; BV: biventricle; CO: cardiac output; EDV: end-diastolic volume; EF: ejection fraction; ESV: end-systolic volume; HR: heart rate; IVCP: inferior vena cava pressure; LAP: left atrial pressure; MBT: modified Blalock-Taussig shunt; NA: not applicable; OP: operation; PAP: pulmonary artery pressure; PVR: pulmonary vascular resistance; SAO_2: arterial oxygen saturation; SV: stroke volume; SVCP: superior vena cava pressure; SVR: systemic vascular resistance (dyn·sec/cm^5). TCPC: total cavopulmonary shunt.

ventricle circulation in pigs (Fig. 1A–C; Video Abstract). The results of the physiological function study are summarized and compared with those of the BV controls (Table 1). The impact of markedly decreased arterial oxygen saturation in the BCPS group (BCPS: 48.8 ± 4.6% vs BV: 99 ± 0.8%, MBT shunt: 80.4 ± 6.5%, TCPC: 99.2 ± 0.8%; $P < 0.01$) was further characterized by significantly lower oxygen saturation in the PA after the surgical procedure compared with the control, the MBT shunt and the TCPC groups. The MBT shunt group had an increased proportion of blood flow directed to the pulmonary circulation through the aortopulmonary shunt, leading to augmented oxygen saturation in the pulmonary artery compared with the BCPS and TCPC groups ($P < 0.01$). In contrast to the MBT shunt and TCPC groups, a significant decrease in haemodynamic oxygen in the BCPS group resulted in a reduced survival time ($P < 0.05$; Table 1).

Bypassing the pulmonary circulation during intravenous injection of cardiosphere-derived cells augmented cell retention in the systemic ventricle

First, we examined the delivery route for CDC engraftment in BV porcine hearts. After intracoronary injection of 9.0×10^5 verified CDCs, the SPIO-labelled cells were readily detectable with MRI and were distributed throughout the entire ventricle (Fig. 2A). Intraventricular delivery of 9.0×10^6 CDCs showed hypointense spots in ventricular muscles, whereas intravenous infusion of the same dose of CDCs revealed few engraftments on the BV hearts (intracoronary: 48.4 ± 8.1%; intraventricle: 47.9 ± 7.7%; intravenous: 1.6 ± 1.1%; $P < 0.001$; Fig. 2A). To determine whether shunt types affect CDC homing in the single ventricle after intravenous administration by avoiding stem cell trafficking to the lungs, SPIO-labelled 9.0×10^6 CDCs were transplanted into pigs with 3 different palliative stages of a single ventricle and compared with BV control hearts [7]. As shown in Fig. 2A, a significant increase in the area of hypointense regions was found in the MBT shunt and BCPS groups compared with the control. In contrast, the TCPC group exhibited a low level of SPIO-labelled CDCs on MRI, which was comparable to those in the BV controls (BV: 1.6 ± 1.1%; MBT shunt: 53.2 ± 9.1%; BCPS: 45.0 ± 9.0%; TCPC: 1.2 ± 0.6%; $P < 0.01$). Histologic sections of the heart were stained with Prussian blue to expose the transplanted CDCs present on the myocardium. Histologic examination showed that the number of SPIO-labelled CDCs was consistent with the MRI results (BV: 0.6 ± 0.6 cells/mm^2; MBT shunt: 4.0 ± 1.8 cells/mm^2; BCPS: 3.4 ± 1.3 cells/mm^2; TCPC: 0.4 ± 0.4 cells/mm^2; $P < 0.01$, Figs, 2B and 2C).

Statistical power and sample size

Power was calculated from the results obtained, which were 0.91 for the low-signal intensity area in the MRI scans and 0.70 for the histologically detected SPIO-labelled CDCs number. In addition, from the effect size calculated based on the experimental data, the desired sample size at a significance level of 0.05% was calculated to be 24 animals for MRI data and 36 animals for the histologic data.

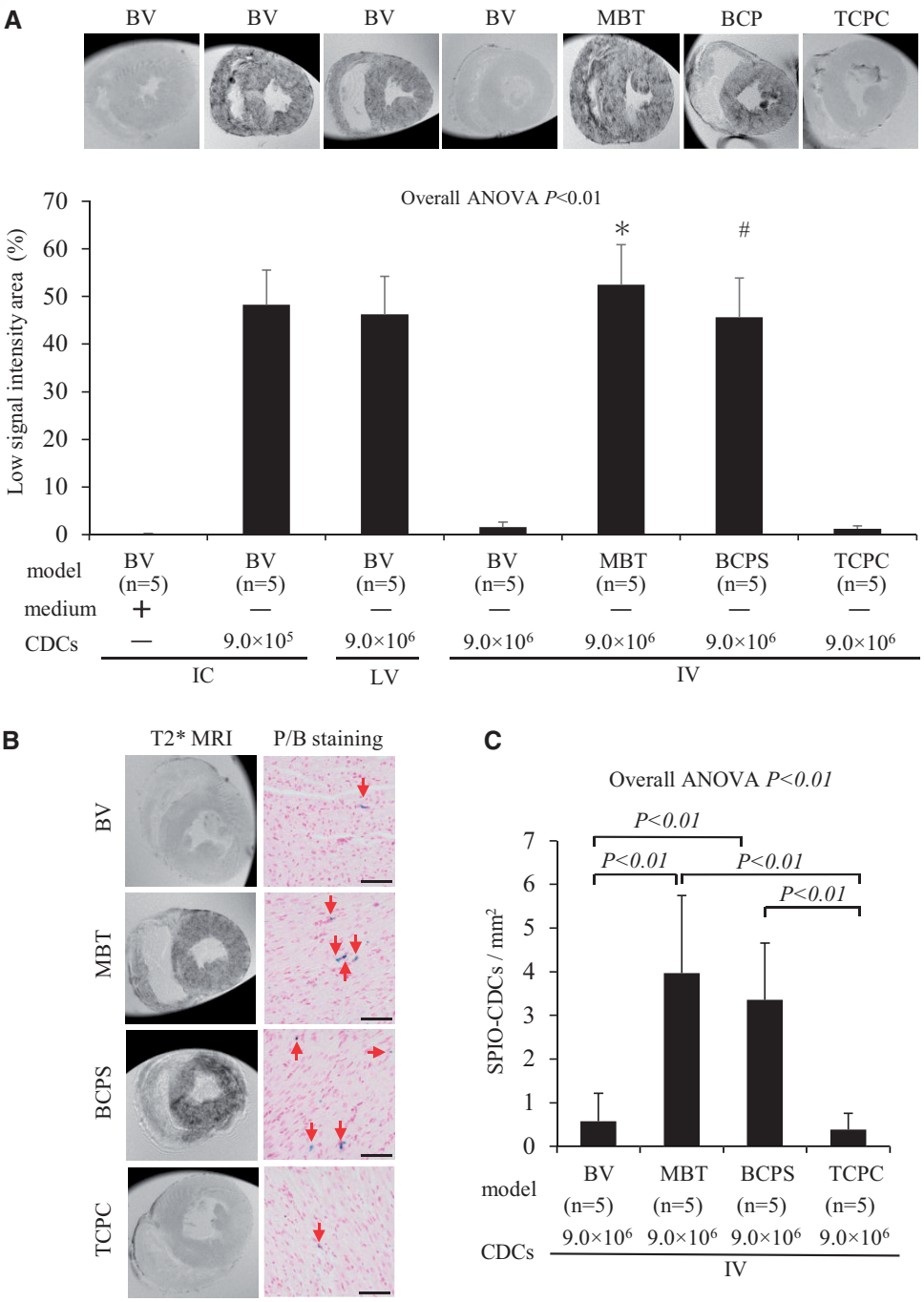


Figure 2: (A) Early cardiac homing after injection of cardiosphere-derived cells for magnetic resonance imaging. Among the intravenously infused groups, the modified Blalock–Taussig shunt and bidirectional cavopulmonary shunt had significantly larger hypointensity areas than those in the normal biventricular group or the total cavopulmonary connection. ANOVA: analysis of variance; BCPS: bidirectional cavopulmonary shunt; BV: biventricle; CDC: cardiosphere-derived cell; IC: ; IV: ; LV: left ventricle; MBT: modified Blalock-Taussig; MRI: magnetic resonance imaging; P/B: ; SPIO: superparamagnetic iron oxide; TCPC: total cavopulmonary connection.

DISCUSSION

Transplanting stem cells has emerged as an adjunctive therapy to enhance staged surgical repair in patients with congenital heart disease [5, 8, 9, 10].

However, cell-based experimental studies in animal models with SVP have never been described because of the technical difficulties in reconstituting univentricular circulation in normally developed postnatal hearts [1, 2]. This study is the first to develop 3 distinct-stage single-ventricle models in pigs: MBT shunt, BCPA and TCPC. Our main finding was that the intravenously administered CDCs were well distributed in the MBT shunt and BCPS models.

Single-ventricle swine model

Creating a chronic, large animal model of SVP remains a significant challenge. Early studies of univentricular circulation have substantially relied on acute experimental models with systemic-to-pulmonary shunt procedures, which have been shown to

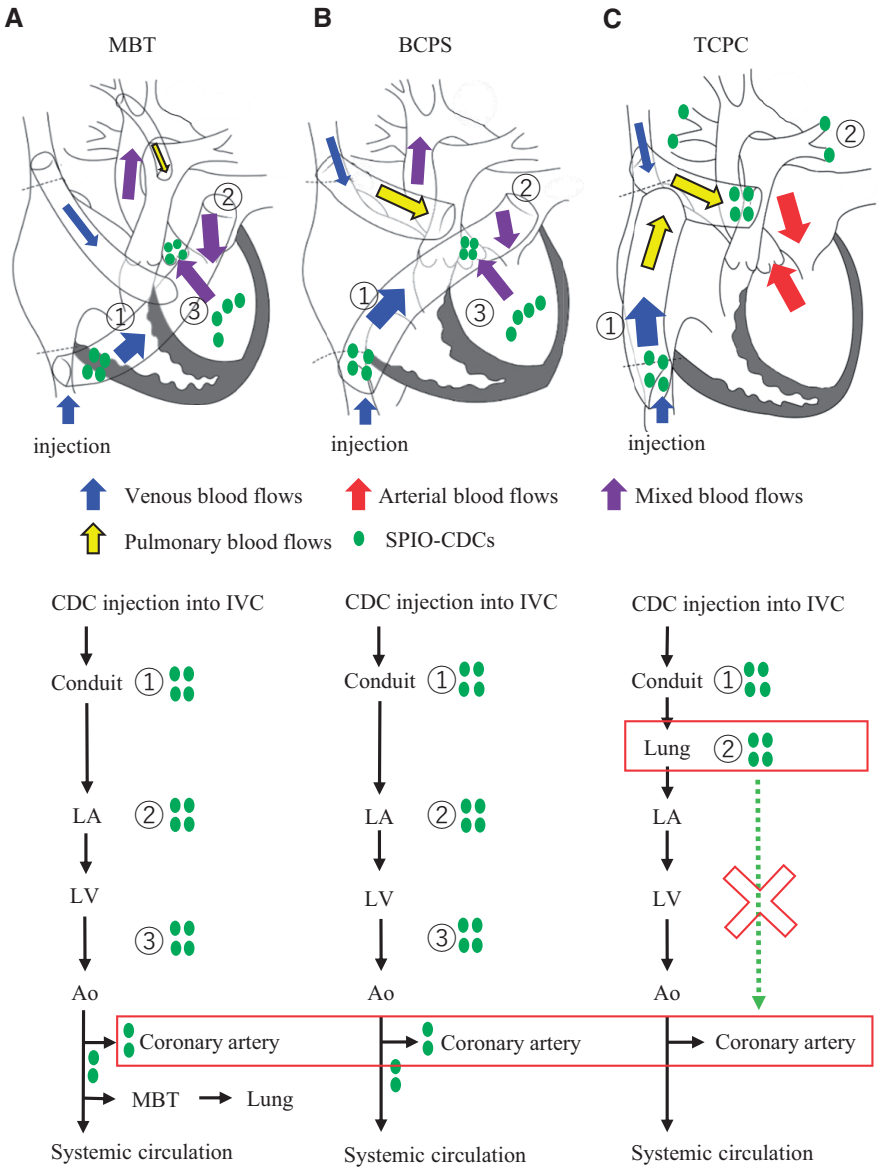


Figure 3. Cardiosphere-derived cells trafficking to the heart in swine models of single ventricular circulation. In the modified Blalock-Taussig shunt and bidirectional cavopulmonary shunt models, intravenously injected cardiosphere-derived cells may entirely fill into the left ventricle and circulate into a systemic blood flow including the coronary artery before supplying pulmonary blood flow (**A** and **B**). In the total cavopulmonary connection model, a subset of cardiosphere-derived cells may circulate into the pulmonary bloodstream through a prosthetic conduit, and most of them are trapped by the lungs before entering the systemic circulation (**C**). In the modified Blalock shunt and bidirectional cavopulmonary shunt models, early cardiosphere-derived cell dispersion in the lungs could be avoided by right heart bypass during the first circulation after intravenous cardiosphere-derived cells infusion. Ao: aorta; BCPS: bidirectional cavopulmonary shunt; CDC: cardiosphere-derived cell; IVC: inferior vena cava; LA: left atrium; LV: left ventricle; MBT: modified Blalock-Taussig; SPIO: superparamagnetic iron oxide; TCPC: total cavopulmonary connection.

induce volume overload [11]. In our experiments, a Y-shaped conduit was used to directly deliver blood flow from both the SVC and IVC into the LA to complete the right heart bypass. The advantage of this novel large animal model is the avoidance of intracardiac surgery requiring cardiopulmonary bypass to achieve right heart exclusion-like physiology. Although the PA was not ligated, the coronary venous return was drained into the pulmonary blood flow, in which high coronary sinus pressure had a negligible effect on LV dysfunction [12].

The haemodynamics of our swine MBT model were similar to those previously reported [13]. Despite the short procedure time, clamping of the brachiocephalic artery in pigs for anastomosis of the artificial graft is controversial. For the anastomosis of a systemic pulmonary shunt, the left subclavian artery may be preferable to avoid brain ischaemia.

An IVC-to-LA shunt was applied to our BCPS model to achieve partial right heart bypass. Although the experimental animals in our study died within 20 h after the surgical procedure, the cardiac output of the LV was maintained by an increased preload, unlike in previous study results [14, 15], both the PA pressure and systemic afterload remained unchanged with a moderate increase in pulmonary vascular resistance.

Experimental Fontan circulation was initially described as the intravenous delivery of a Y-shaped conduit constructed using polyethylene cannulas, without the use of cardiopulmonary bypass [16]. In our TCPC model, extracardiac conduit anastomoses

were performed to directly connect both the SVC and IVC to the PA. However, all 3 models exhibited very short survival times. The causes of short survival times in these models seemed to differ. For the MBT model, volume overload to the LV was the main reason; for the BCPS model, low pulmonary blood flow and desaturation were critical; and for the TCPC model, intravascular volume loss with ascites retention led to circulatory failure. To control the pulmonary-to-systemic flow ratio, heart failure management and infusion therapy to maintain intravascular fluid seemed to be the keys to increase the models' survival times.

The left azygos vein in pigs differs from that in humans, which functions as a systemic vein to an atrial shunt. Therefore, total right heart bypass in the strict sense was incomplete in this study's model. Intravenously injected stem cells were tracked in the presence of the left azygos vein. Ligating the left azygos vein without cardiopulmonary bypass is technically difficult and carries the risk of bleeding. The BCPS model was considered the most affected by the presence of the left azygos vein. It is believed that a systemic venous-atrial shunt, which humans do not normally have, leads to lower blood oxygen levels.

CDC Dosage

The number of CDCs administered was based on data from a previous study, but if it was exactly equivalent to that of the previous study [5], it would have been better to increase the dose to the coronary arteries by 10 times (300,000 cells/kg) compared to this study. However, the piglets weighed 5–10 times more than those used in previous human study [5], making it impractical to culture sufficient cells. Therefore, the intravenous dose was set at 300,000/kg, and the coronary artery dose was set at 30,000/kg. However, this reduced dose of the cells did not undermine the purpose of tracking the destinations of the injected cells.

Impact of administration technique for cell therapy

Recent clinical and preclinical studies have shown that the delivery route of the cells may potentially affect ventricular function after infarction. However, many aspects remain unclear, including the appropriate cell dose for injection and the efficient route of stem cell administration in patients with SVP [17]. To our knowledge, this is the first preclinical study to demonstrate the differential cardiac homing dynamics of transplanted CDCs in experimental models of SVP.

Regarding the cardiac homing of stem cells, the inefficiency of intravenous injection was more obvious than that of intracoronary injection. However, the non-invasiveness of the administration method may enable repeated administration of stem cells and overcome this inefficiency. Furthermore, this delivery approach may offer several advantages, including repeated cell infusion to elicit salubrious effects cooperatively through paracrine/endocrine mechanisms of intrinsic and extracardiac origin [18]. In our MBT shunt and BCPS models, intravenously delivered CDCs can be diverted into the coronary arteries during initial circulation by bypassing the pulmonary circuit in a systemic blood flow-dependent manner to enhance acute cell retention (Fig. 3). As anticipated, preferential engraftment in the lungs and other extra-cardiac organs may subsequently occur during circulation. It is conceivable that endogenous cardiomyocytes and stem/progenitor cells can be stimulated directly by transplanted CDCs to proliferate and that anti-inflammatory responses and cell protection signaling may be provoked by the CDCs at the site of their systemic delivery [19].

Limitations

Although this study was the first to develop chronic models of SVP, haemodynamic instability after the surgical procedures limited this work to an acute study with a short survival period, which may restrict further studies to investigate stage-specific functional outcomes. The small sample size was a statistical limitation because there was insufficient power to demonstrate the anticipated effects.

CONCLUSIONS

Three-stage right heart bypass surgery was feasible with durable outcomes to study early cardiac homing of the transplanted CDCs with haemodynamic variables compatible with the single LV. Additionally, CDC delivery through the lung bypass circuit is effective in targeting a single ventricle before TCPC completion.

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DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

Author Contributions

Takuya Goto: Conceptualization; Data curation; Investigation; Writing—original draft. **Daiki Ousaka:** Conceptualization; Data curation; Investigation; **Kenta Hirai:** Data curation; **Yasuhiro Kotani:** Supervision; Writing—review & editing. **Shingo Kasahara:** Supervision; Writing—review & editing.

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