Transcoronary cell infusion with the stop-flow technique in children with single-ventricle physiology

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Abstract

Background: Almost all reports on cardiac regeneration therapy have referred to adults, and only a few have
focused on transcoronary infusion of cardiac progenitor cells using the stop-flow technique in children.

Methods: Intracoronary autologous cardiosphere-derived cell (CDC) transfer was conducted at Okayama University as
a phase 1 clinical trial for seven patients with hypoplastic left heart syndrome between January 2011 and December 2012, and as a phase 2 clinical trial for 34 patients with single-ventricle physiology between July 2013 and March 2015.
Results: A total of 41 patients with single-ventricle physiology underwent transcoronary infusion of CDC with the stop-flow technique. The median age was 33 months (range, 5–70 months) and the median weight was 10.1 kg(range, 4.1–16.0 kg). Transient adverse events occurred during the procedure, including ST-segment elevation or depression, hypotension, bradycardia, and coronary artery vasospasm. All patients completely recovered. There were no major procedure-related adverse events. In this study, transcoronary infusion of CDC using the stop-flow technique was successfully completed in all patients.

Conclusion: Transcoronary infusion of CDC using the stop-flow technique in children is a feasible and safe procedure.

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Key words: stop-flow technique, transcoronary infusion, congenital heart disease, regeneration therapy, temporary occlusion balloon

Introduction

From January 2011 to March 2015, Okayama University Hospital conducted clinical trials on autologous cardiac progenitor cell transplantation. The safety of this technique was confirmed in a phase 1 clinical trial, and a random comparison was carried out in a phase 2 clinical trial.[1,2)]

In terms of the clinical procedure, the most important aspect is the infusion of cultured cardiosphere-derived cells (CDCs) via the distal coronary artery, which has been blocked with a temporary occlusion balloon; however, to date, there have been almost no comprehensive reports on the use of this procedure in children. We investigated the procedure for the transcoronary infusion of CDCs by using coronary artery balloon occlusion (the “stop-flow technique”) in children, including the safety of this procedure and the occurrence of adverse events.

Subjects

The study subjects comprised a total of 41 children treated at our hospital. Seven of these children were patients with hypoplastic left heart syndrome (HLHS) who underwent autologous CDC transfer through transcoronary infusion with the stop-flow technique in a phase 1 clinical trial between January 2011 and December 2012. The remaining 34 children were patients with single-ventricle physiology who underwent this procedure in a phase 2 clinical trial between July 2013 and March 2015.

Methods: Transfer Procedure

1) Cell harvest

In both the phase 1 and phase 2 clinical trials, a section of the right atrium wall was harvested during second-stage surgery (Glenn procedure) or third-stage surgery (Fontan procedure), both of which were elective surgeries. CDCs were subsequently isolated from the harvested tissue and cultured.

2) Timing of autologous CDC transfer

In both the phase 1 and phase 2 clinical trials, transcoronary infusion of CDCs was performed around 1 month after the tissue harvest operation (i.e., Glenn procedure or Fontan procedure).

3) Cell validation

The CDC cultures were prepared such that on the day of autologous CDC transfer, the cell count was 3.0×10^5 cells/ kilogram body weight in a total volume of 3 mL culture solution.

4) T**ranscoronary infusion**

The procedure was performed in a catheterization laboratory equipped with a biplane cardiovascular imaging system (INFX-8000V Biplane; Toshiba, Tokyo, Japan). Under general anesthesia,the patients were monitored with surface 12-lead electrocardiography (ECG), pulse oximetry, and invasive blood pressure measurements. Heparin (100 units/kg) was initially given i.v. at the start of the procedure, and the dose was subsequently adjusted as necessary to maintain the activated clotting time at 200–250 s. After the completion of pressure measurement, blood sampling and ventriculography through regular cardiac catheterization were conducted. Amiodarone (10 lg/kg/min) was given i.v. for approximately 30 min before transcoronary CDC infusion, to prevent the procedure from causing serious arrhythmia.

A 5F Launcher guiding catheter (Medtronic, Minneapolis, MN, USA) was passed through a 5F sheath placed in the femoral artery. Although a Judkins right coronary type catheter was usually used, when deemed necessary, either a catheter with a different curve was chosen or a steam-shaped guiding catheter was used. After its introduction through the femoral artery, the guiding catheter was fixed either in the proximal coronary artery or at the coronary cusp, and coronary angiography was performed. Care was taken not to advance the guiding catheter into the coronary artery, to prevent wedge and blocking of the coronary perfusion. The decision on how to divide the 3 mL CDC solution for infusion was made on the basis of coronary angiography: in typical cases, 1 mL each was infused into the right coronary artery, anterior descending branch, and circumflex branch (Fig. 1).

As backup for the 5F guiding catheter that had been shaped as necessary, a 0.012-in GT guidewire (Terumo, Tokyo, Japan) into which a 2.8F temporary occlusion balloon (Iiguman type C in the phase 1 trial, Kudos in the phase 2 trial; both from Fuji Systems Corporation, Tokyo, Japan) had already been inserted was introduced as far as the target site in the coronary artery. Iiguman is a dual-lumen occlusion balloon designed for perfusion control during transcatheter arterial embolization or ethanol embolization of the liver or kidneys; but, because the length of the balloon was deemed too long at 10 mm, Kudos, which has a length of 5 mm, was instead developed by the manufacturer at our request and was used in the phase 2 trial.

To start the procedure, nitroglycerine 0.5–1.0 lg/kg was infused into the coronary artery at the target site from the tip of the Kudos balloon (Fuji Systems, Tokyo, Japan) during a period of at least 1 min, with careful attention to the occurrence of hypotension. The balloon was then manually dilated using a 5:1 dilution of contrast agent, stopping the coronary perfusion. While the blood flow was halted, 1 mL CDC was infused in a single injection and washed through with physiological saline (transcoronary infusion of CDC with the stop-flow technique).

In the phase 1 trial, we intended to block the coronary perfusion for approximately 2 min, but many patients could not tolerate a 2 min interruption in blood flow because of hypotension or ST-segment changes on ECG (ST elevation or depression >1–2 mm at horizontal line) or bradycardia. Thus, in the phase 2 trial, this duration was reduced to approximately 1 min, and we established the balloon deflation criterion as follows: the balloon was to be deflated immediately in the event of a drop in blood pressure >15 mmHg or a decrease in heart rate >20 beats/min. Once the coronary perfusion block had been released and it had been confirmed that blood pressure and ECG had returned to normal, contrast enhancement was performed via the Kudos balloon (Fuji Systems, Tokyo, Japan) to check for coronary artery vasospasm. If vasospasm had occurred, nitroglycerine was again infused (Fig. 2) and the resulting improvement was confirmed. The Kudos balloon (Fuji Systems, Tokyo, Japan) was then moved to a different coronary artery target site, and the same procedure was repeated. Coronary angiography was again performed to check for vasospasm after the entire 3 mL CDC (3 9 105 cells/kg) had been infused. Once it had been confirmed that no vasospasm was present, the procedure was deemed completed.

5) Post-procedure monitoring

After extubation in the catheter laboratory, if the patients' vital signs were stable, they were returned to the ward and monitored with pulse oximetry and ECG as well as noninvasive blood pressure measurement every 3 h. On the day after the treatment, pulse oximetry and ECG monitoring were continued and blood pressure was measured noninvasively three times a day. Two days later, the monitors were removed and vital signs were measured three times a day. Continuous infusion of amiodarone (AMD)10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was continued until the following morning, when it was possible to switch to oral AMD 2.5 mg/kg. Oral AMD 2.5 mg/kg was administered twice daily for 3 days, after which it was discontinued. However, if the patients' heart rate was inappropriately slow for their body weight, both infusion and oral administration were discontinued and were not restarted.

Results

1) Patient characteristics (Table 1)

The 41 patients consisted of 21 boys and 20 girls who underwent autologous CDC transfer through coronary infusion at a median age of 33 months (mean, 33 _ 17 months) and a median bodyweight of 10.1 kg (mean, 10.0 _ 3.0 kg; Table 1). The primary condition was HLHS (including variant type) in 22, asplenia/single ventricle/common atrioventricular valve in seven, tricuspid atresia type IIc (i.e., transposition of the great artery and ventricular septal defect without pulmonary stenosis) in two, polysplenia/single ventricle in two, double-outlet right ventricle/hypoplastic left ventricle in two, single left ventricle in two, and other conditions in four patients. The transfer was performed after the Glenn procedure in 10 patients and after the Fontan procedure in 31.

2) Adverse events (Table 2)

Transient ST changes on ECG were evident in 39 of the 41 patients (95%), and drop in blood pressure >15 mmHg in 18 (44%), decrease in heart rate >20 beats/min in five (12%), and transient coronary artery vasospasm in eight patients (20%) were also observed (Table 2). In the case of ST-segment changes occurring alone, the patients' condition was monitored. In contrast, for drops in blood pressure or heart rate, in the phase 2 trial, the criterion was set as described here (i.e. the balloon was to be deflated immediately in the event of a drop in blood pressure >15 mmHg or a decrease in heart rate >20 beats/min). Coronary artery vasospasm was treated with nitroglycerine. The shortest duration of coronary occlusion was 5 s and the longest was 163 s, with a median duration of 55 s. There were no deaths or serious complications such as ventricular fibrillation (VF) or acute myocardial infarction (AMI), indicating that the procedure was safe.

3) Coronary artery and major vessels

There were three major morphological patterns in the anatomical relationship between the coronary artery and major vessels, and these required attention during the procedure. The choice of the guiding catheter and its shaping and manipulation depended not only on detailed angiography, but also on contrast-enhanced computed tomography (CT) before the procedure.

The first pattern was the narrow native aorta and single coronary artery seen in HLHS, which was present in 24 patients (59%; Fig. 3). In these patients, if the guiding catheter was engaged in the proximal area, the left and right branches could be accessed simply by manipulating the Kudos balloon (Fuji Systems, Tokyo, Japan) and the 0.012 in. guidewire; but if the guiding catheter was advanced further than necessary, then the entire coronary artery was restricted and care was therefore required not to place the guiding catheter in too deep a position. In the second pattern, which occurs in cases of single ventricle and pulmonary atresia, the left and right coronary arteries originate separately from the enlarged aorta; this pattern was present in seven patients (17%; Fig. 4). In those patients, the guiding catheters had to be selected and shaped appropriately for separate insertion into the left and right coronary arteries. The third pattern was the coronary artery pattern seen after the Damus-Kaye-Stansel procedure, which was present in 10 patients (24%; Fig. 5). In those patients, the appropriate position of the guiding catheter was frequently difficult to determine. When the pulmonary artery is in the anterior position and the aorta is in the posterior position, it was particularly difficult, because the backup force on the side of the greater curvature was unobtainable. Thus, it was sometimes necessary shape the catheter to enable backup to be obtained on the side of the lesser curvature of the aorta.

4) Therapeutic outcomes

As previously reported in the phase 1 clinical trial, the right ventricular ejection fraction (RVEF) in the seven patients with HLHS improved significantly on magnetic resonance imaging (MRI) from 46.9 _ 4.6% before the procedure to 54.0±2.8% after the procedure,

and this improvement has been maintained for 3 years.1,2 Analysis of data from the phase 2 clinical trial is currently under way.

Discussion

A wide variety of cells can be used in myocardial regeneration therapy, including skeletal muscle myoblasts, myeloid stem cells, cord blood stem cells, peripheral blood stem cells, and mesenchymal stem cells. To date, >5000 stem cell transplant treatments have been performed worldwide.[3–5] In 2004, Messina *et al.* [6] successfully isolated undifferentiated cells from human cardiac atrial and ventricular tissue that can be cultured by adhesion, and named them "cardiospheres". In 2007, Smith *et al.* [7] established a method for culturing stem cells in sufficient quality for clinical use from heart muscle biopsy samples, and named these cells "cardiosphere-derived cells" (CDC). Subsequent studies have found that CDC are abundantly present in the right atrium and right ventricle, that these cells are present in greater numbers in the heart muscle of children than in adults, that the CDC found in children have higher regenerative potential than those of adults, and that CDC can be reliably isolated and cultivated from cyanotic children with congenital heart disease.[8,9] When we focused on CDC with these attributes for use in these clinical trials, we found that it was possible to reliably isolate and cultivate CDC at $>3 \times 10^5$ cells/kg during a 4-week period from right atrial tissue harvested during surgery.

Cell transfer methods include i.v. treatment, intracardiac myocardial infusion, open intra-arterial infusion, and percutaneous coronary artery infusion. Reopening the chest to transfer stem cells would be highly invasive for children who had recently undergone second- or third-stage surgery, and transcoronary infusion with the stop-flow technique has been reported to be clinically effective. Therefore, we decided to use the stop-flow technique instead.[10–13]

The SPICIO and CADUCEUS trials were two comparative large-scale randomized clinical trials of percutaneous coronary infusion of stem cells in adult patients with old myocardial infarctions(MI) [10,11]. The stem cells used were c-kit-positive cardiac stem cells in the SPICIO trial and CDC in the CADUCEUS trial, and the coronary artery that had caused the infarction was closed with a balloon for 3 and 15 min during infusion, respectively. In the SPICIO trial, left ventricular EF (LVEF) improved by 8.2%[10], and although no improvement was evident in the CADUCEUS trial, the fibrotic area did contract[11], with the procedure safely performed in both trials. In those studies, the infarction site was in the target coronary perfusion region; therefore, it can be inferred that even the occlusion of coronary blood flow for a comparatively long time had little effect on hemodynamics. In the present patients, however, the target was not a calcified coronary artery or infarction site but rather the myocardial region, which is still in constant motion. Coronary artery occlusion with the stop-flow technique may thus have a greater effect on hemodynamics than that seen in adults in the aforementioned studies. In consideration of the possible adverse effects of coronary balloon occlusion, in the phase 2 trial, we set the criterion that the balloon was to be deflated immediately in the event of a drop in blood pressure >15

mmHg or a decrease in heart rate >20 beats/min, and this practice is important for the safe implementation of this technique.

In terms of other reports on myocardial stem cell infusion with the stop-flow technique in children, Limsuwan et al.12 carried out percutaneous transcoronary infusion of autologous myeloid stem cells in an 8-year-old girl who had MI. An over-the-wire 2.5 9 12 mm coronary artery balloon deployed in the left coronary artery was inflated and maintained at a pressure <2 atm for 3 min to block coronary perfusion, and a total cell volume of 20 mL was infused in four increments. After the procedure, LVEF on MRI improved from 31.4% to 47.9% and the area of fibrosis decreased from 50% to 41%, allowing avoidance of heart transplantation. Rupp et al.13 treated nine children (three with coronary heart disease and six with dilated cardiomyopathy), aged between 4 months and 16 years, by means of percutaneous transcoronary infusion of myeloid stem cells while inflating an over-the-wire coronary artery treatment balloon (width 1.5–2.5 mm, length 8–20 mm) and maintaining it at a pressure of 1–2 atm for 3 min. Although moderate ST-segment changes were observed, the procedure was implemented safely, with no troponin T elevation observed in any cases. Three of their patients received heart transplants and one patient died; in the other five patients, however, RVEF improved from 24% to 41%, and New York Heart Association score and B-type natriuretic peptide also decreased. Until the present study on transcoronary infusion of CDC with the stopflow technique in 41 children, the Rupp et al.13 study had involved the largest number of pediatric cases.

In the present study, we also observed no serious complications such as VF or AMI, and the safe performance of this procedure further shows that transcoronary infusion of CDC with the stop-flow technique can be safely completed in children.

In their experiment on pigs, Suzuki et al.14 found that the effect of global infusion without blocking coronary perfusion was not inferior to that of the stop-flow method if the number of CDC was increased by approximately threefold. This method has also been clinically established, and if the adverse events associated with the larger number of CDC can be overcome, then it may be possible to eliminate the risks associated with coronary balloon occlusion.

From the procedural standpoint of the great vessels, we classified the anatomical relationship between the coronary artery and the major vessels into three morphological patterns. The most common of these was the first pattern, which was characterized by a narrow native aorta and a single coronary artery. The reason was that the majority of patients had HLHS (n = 22; 54%). We also had two cases of single coronary artery. As noted, the selection and design of the guiding catheter are currently determined with reference to angiography and contrast-enhanced CT obtained before the procedure. Although we did not perform 3-D rotational angiography in the present study, there have been more reports on congenital heart disease recently, and this is a topic for future investigations.[15)]

Conclusion

We performed CDC transfer with the stop-flow technique in 41 children with single-ventricle physiology. Although ECG changes, hypotension, bradycardia, and coronary artery vasospasm occurred during the procedure, these were transient and resolved in all cases, and none of the patients developed serious complications. The procedure was safely completed in all cases. Transcoronary infusion of CDC with the stop-flow technique can be safely performed in children with single-ventricle physiology.

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Author Contributions

Hidemasa Oh designed the study. Shinichi Ohtsuki, Kenji Baba, Takahiro Eitoku, and Maiko Kondou performed intracoronary injection. Shunji Sano and Shingo Kasahara performed surgical operation and received right atrium tissues. Shuta Ishigami and Kenta Hirai harvested CDCs. Yoshihiko Kurita and Yousuke Fukushima provided conceptual advices.

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