Improvement of Local Cell Delivery Using Helix Transendocardial Delivery Catheter in a Porcine Heart

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SUMMARY

Cardiac regeneration strategies using stem cells have shown variable and inconsistent results with respect to patient cardiac function and clinical outcomes. There has been increasing consensus that improving the efficiency of delivery may improve results. The Helix transendocardial delivery system (BioCardia Inc.) has been developed to enable percutaneous transendocardial biotherapeutic delivery. Therefore, we evaluated cell retention using this unique system compared with direct transendocardial injection and intracoronary infusion in an animal model.

Twelve healthy swine were used in this study. 18Fluorodeoxyglucose (FDG)-labeled bone marrow mononuclear cells were delivered via percutaneous transendocardial route using the Helix system (TE group, \( n = 5 \)), via direct transendocardial injection using a straight 27-gauge needle in an open chest procedure (TP group, \( n = 4 \)), or via percutaneous intracoronary (IC) infusion (IC group, \( n = 3 \)). One hour after cell delivery, the distribution of injected cells within the myocardium was assessed by PET-CT. Regions of interest were defined and their signals were compared in each group. Retention rates were calculated as a percentage of the comparing signal.

The distribution of injected cells in the myocardium was higher in the TE group (17.9%) than in the TP group (6.0%, versus TE, \( P < 0.001 \)) and the IC group (1.0%, versus TE, \( P < 0.001 \)). Consistent with previous reports, there were signal distributions in the lungs, liver, and kidneys in qualitative whole body PET assessment.

TE cell delivery using a helical infusion catheter is more efficient in cell retention than either TP delivery or IC delivery using PET-CT analysis. (Int Heart J 2017; 58: 435-440)

Key words: New device, Percutaneous, Transendocardial, Cell retention, PET-CT, Stem cells

The leading cause of death and healthcare expenditure in the United States is due to cardiovascular-related diseases.11 Myocardial infarction size is related with inhospital and long-term mortality and morbidity.22 Although the mortality rate for acute myocardial infarction has improved dramatically with advancements in pharmacotherapy and interventional treatments, these treatments are only aimed at slowing down the disease and have limited or no benefits towards reducing infarct size. Stem cell therapy has emerged as a new paradigm of treatment with potential regenerative capabilities and a wide range of applications in medicine.34 A recent meta-analysis of stem cell therapy trials for chronic ischemic heart disease and congestive heart failure comparing bone marrow mononuclear cells (BMNCs) to placebo control or optimal medical treatment, showed evidence of reduction in mortality and rehospitalization due to heart failure at long term follow-up.3 Functional measures including left ventricular end systolic volume, stroke volume index, and left ventricular ejection fraction (LVEF) as well as New York Heart Association class all showed significant improvements in favor of BMNCs at short term follow-up. However, although previous clinical studies with BMNCs have consistently been shown to be safe, the level of improvement of left ventricular (LV) functional measures has been inconsistent across trials. This may be due to variable study designs with different cell doses, injection schedules, and delivery routes.89

One method to improve patient benefit is to optimize the delivery method for ease of use, safe delivery, and enhanced retention and engraftment of cells. Different approaches have been used for delivering cells to the heart, including infusion via coronary artery (IC), systemic vein (IV), retrograde coronary sinus, and direct intramyocardial (IM) injection with transcardiac and transendocardial approaches, and cell sheet technology.10 Selection of the best cell delivery route is vital and dependent on the disease indication to be treated as it affects cell retention, survival, and engraftment in the heart.
Comparative studies of the efficacy of cell delivery among IV, IC, and IM have reported different results\(^9\,^{10}\) and the optimal method for cell delivery is still being debated. Lower than expected retention and engraftment after direct IM injection may be due to back-leak of the injected cells through the entry holes into the pericardial space during myocardial contraction,\(^1\,^{13}\) which has also been shown with transcendocardial delivery. However, direct IM injection is an invasive approach as it requires access to the epicardium via the open chest and the pericardial sac and is therefore usually performed during coronary artery bypass graft (CABG) and open chest surgery. For this reason, transcendocardial access to the myocardium using the minimally invasive technique of percutaneous intervention has become more and more important over the past decade.

The Helix transcendocardial delivery catheter (the Helix) together with the Morph Universal Deflectable Guide Catheter (BioCardia Inc., San Carlos, California) has been developed to improve delivery of biotherapeutic agents via percutaneous transcendocardial intramyocardial (TE) injections.

In this study, we assessed the cell retention after TE injection using this unique delivery system compared to conventional transendocardial intramyocardial (TP) injection and IC infusion in the swine model.

**Methods**

**Animal model:** Twelve healthy female Yorkshire swine (8-12 weeks old, 40-60 kg) were used in this study. The animals were anesthetized via an intramuscular injection of Telazol (6 mg/kg). Pancuronium (0.1 mg/kg) was administered IV just prior to the start of the procedure at the veterinarian’s discretion to decrease muscle spasm. After intubation, anesthesia was maintained with 2.0% isoflurane with oxygen during the procedure of cell delivery. This study was reviewed and approved by the Animal Research Committee at the animal facility, Surpass Inc. (formerly LyChron LLC., Mountain View, CA) and complies with all applicable Federal and State laws, guidelines, and regulations governing the use of animals in instruction and research and uses the provisions in the PHS/NIH Guide for the Care and Use of Laboratory Animals as a guideline.

**Cell preparation:** Fifty milliliters of autologous bone marrow cells were collected under local anesthesia from the swine posterior iliac crest. BMNCs were isolated by density gradient using Ficoll-Paque Plus (GE Healthcare, Tampa, FL, USA) and the cells were resuspended in serum-free phosphate buffered saline (PBS) solution for \(^{18}F\)-FDG radiolabeling. The labeling protocol was as previously described.\(^1\,^{15}\) The isolated cells were incubated in a 15 mL conical tube with 100 MBq \(^{18}F\)-FDG (specific activity, 10 MBq/mL) for 30 minutes at room temperature with gentle rocking in 10 mL serum-free PBS (pH 7.2) containing 10 U/mL heparin and 0.1 U/mL recombinant human insulin and washed 3 times in 10 mL heparinized PBS at 1000 rpm, 2 minutes each. Radioactivity in the supernatant and cell pellet was measured with a dose calibrator. The \(^{18}F\)-FDG-labeled cell pellet was resuspended in 1-2 mL heparinized Normosol-R for TE and TP injections and in 10 mL heparinized Normosol-R for IC infusions. Cell viability was assessed by trypan blue dye exclusion assay before injections.

**Cell delivery methods:**

**TE delivery** The Helix is an injection catheter with a helical needle that enables accurate and controlled delivery within the myocardium (Figure 1A). The Morph Universal Deflectable Guide Catheter is an 8-French guide catheter that enables significant deflection of its distal end and provides the ability to guide the Helix into all areas within the left ventricle (Figure 1B). The Morph Universal Deflectable Guide catheter was advanced into the LV over a guide wire under fluoroscopic guidance and the wire was removed to allow for advancement and navigation of the Helix (Figure 1C). The cells were delivered to the left anterior descending (LAD) coronary artery region using coronary angiography and left ventriculography under biplane fluoroscopic guidance. One hundred million \(^{18}F\)-FDG-labeled BMNCs were injected for a total of 6 injections (0.1 mL per injection) into the heart.

**TP delivery** After median sternotomy, the pericardium was opened to expose the anterior surface of the heart. A 1 mL syringe was connected to a 27-gauge straight needle via a plastic tube. TP injections were performed, keeping the needle at a 90 degree angle and 3 to 4 mm depth using a needle holder in the beating heart by one operator and injecting the cells by another operator in 5-10 seconds. Six injections of 0.1 mL per injection for a total of 100 million \(^{18}F\)-FDG-labeled BMNCs were performed evenly in the anterior wall of the beating heart, along both sides of the LAD coronary artery region.

**IC delivery** After cannulation of the left coronary artery, an over-the-wire angioplasty balloon (Maverick \(\rightarrow\) Over-the-wire coronary dilatation catheter, Boston Scientific, Maple Grove, MN) was inflated distal to the first diagonal of the LAD with adequate pressure to block distal blood flow completely. Intra-coronary infusion of radiolabeled BMNCs was performed following the stop-flow method as previously described.\(^1\) A total of 100 million \(^{18}F\)-FDG-labeled BMNCs were infused (3 mL per infusion) distally through the central port of the occluding balloon catheter during 3x 3-minute balloon inflation. The balloon was deflated between each infusion, coronary artery blood flow was restored, and the myocardium was reperfused for 3 minutes. After the third infusion, coronary angiography was performed to assess the target vessel flow.

**Cardiac PET-CT analysis:** One hour after cell injections, PET-CT images were acquired using a GE Model VCT 64 slice PET/CT scanner (GE Healthcare, Waukesha, WI, USA). The low-dose, attenuation correction CT scan was then acquired over less than 5 seconds. Immediately following CT, dose information was entered into the PET protocol parameters based on initial FDG dose (in mCi) prior to injections, time of injections, and residual activity post injections. A one-bed, 10 minute 3D PET acquisition was taken over the same region as the CT. Data were processed using GE Advanced Windows Workstation Software, Version 4.4. A 3D Maximum Intensity Projection, PET, CT, and fused PET/CT images were viewed and volumetric regions of interest (ROI) were placed over injection sites as well as over the standard. Upon drawing a volumetric ROI over an injection point, values were automatically provided for maximum and average percentage of counts. The threshold intensity was set to include the full injection volume, including any injection demonstrating the smallest visual activity, but not extended beyond the visual PET activity. The maximum and average count values were given as a percentage of the large volume. The standard maximum count value was
100%. The values for maximum count percentage of the standard, average counts, and volume of each ROI were recorded. Regions of interest were defined and their signals compared to a positive control (one dose of cells read during the same PET acquisition process). Retention rates were calculated as a percentage of positive control signal.

**Statistical analysis:** Statistical analysis was performed using SPSS, version 22.0 (SPSS Inc., Chicago, IL). All continuous data are given as the mean ± standard deviation. One-way analysis of variance (ANOVA) with the Tukey test for multiple comparisons was used in continuous variables. A two-tailed $P$-value ($P$) less than 0.05 was considered statistically significant.

**RESULTS**

$^{18}$F-FDG-labeled BMNCs were delivered via TE injection using the Helix ($n = 5$), via TP injection ($n = 4$) with a 27 gauge straight needle in open chest, or IC infusion ($n = 3$) successfully in healthy swine. Approximately 3 times more cells were retained after TE injection (17.9 ± 3.1%) compared to TP injection (6.0 ± 1.5%). The retention rate after IC infusion (1.0 ± 0.8%) was lowest among the 3 groups ($P < 0.001$ in ANOVA; TE versus TP, $P < 0.001$; TE versus IC, $P < 0.001$; TP versus IC, $P = 0.04$) (Figure 2-3). There were some fractions of the delivered cells in the cardiac lymph node in both the TE and TP groups, but no signals in the IC group. Further signal distributions were found in the lungs, liver, and kidneys in qualitative whole body PET assessment, as previously reported. During the procedure, there were no specific ECG changes such as ST-T wave abnormalities and significant arrhythmia. In addition, there were no critical events during the procedure of cell delivery and image acquisition.

**DISCUSSION**

Regeneration therapy in ischemic heart disease represents one of the most promising strategies of late and has generated a wealth of researcher interest. However, in previously reported studies, these highly demanding procedures resulted in modest improvements in LVEF, infarction size, and other cardiovascular functional outcomes and some studies failed to show any significant benefit in their primary outcomes. Other studies have reported dose-dependent effects of cell transplantation, and consequently, the ability of targeting and delivering cells efficiently to the area of interest in the myocardium is very important to reach the expected functional and clinical goals. The meta-analysis of Jeevanantham, et al reported that injection of higher doses in excess of $40 \times 10^6$ BMNCs resulted in significant improvement in LVEF, infarction size, LV end-systolic volume, and LV end-diastolic volume, whereas injection of less than $40 \times 10^6$ BMNCs did not show improvement in any outcome. In a study by de Jong, et al, patients treated with an infusion of less than $100 \times 10^6$ cells did not benefit more or less from cell infusion compared to patients with higher cell doses. IV administration is a simple and physiologic process but has very low efficiency when applied in animal and clinical studies. Hou, et al reported that the retention efficacy of IC administration using an infusion balloon catheter has overcome many issues of IV methods, yet in their study, only 2% of the stem cells infused via IC route was reported to reach the infarct and peri-infarct zones in the swine model. Several clinical trials have used IC routes for patients following acute myocardial infarction because most interventional cardiologists are familiar with this approach and it has been hypothesized that the cells will tend to migrate towards the infarct site as a response to fresh injury. Many trials using...
IC routes found results inconsistent and of note, the Cardiovascular Cell Therapy Research Network’s randomized TIME trial and LateTIME trial found that IC infusion of autologous BMNCs did not improve LV function compared with placebo. This could be due to its low retention rate in the heart because of the rapid loss of a high proportion of cells into the systemic circulation within a few minutes, and high systemic organ deposition after IC infusion.

The alternative method to overcome the limitations of IC infusion is direct IM injection via open chest or percutaneously. Surgical IM injection has the advantage of injecting cells into the myocardium under direct visualization. However, this method also has several limitations, such as high surgical risk and would be restricted only to patients who are supposed to receive a thoracotomy such as CABG. If surgical IM injection was performed using a minimal incision, there would be technical limitations injecting into all walls of the left ventricle and especially the posterior or lateral walls as access would be

**Figure 2.** Representative PET/CT images of a cardiac slice in swine after transendocardial injections, transepicardial injections, and intracoronary artery infusion. Top row: 1: Control (100 µL of 18F-FDG labeled BMNCs in a microcentrifuge tube taped on the right side of the chest of the swine), 2: cardiac lymphatic node, 3-8: injections in the myocardium of the left ventricle. The PET-CT signals for the injections were calculated relative to the control marked as 100% to account for the decay of the radioisotope and the cell volume retention was thus calculated.

Bottom row: Cardiac PET-CT signals after transendocardial injections, transepicardial injections, and intracoronary infusion in swine. The signals as they appear after the PET-CT scans are shown. Each injection site is identified and the region of interest (ROI) is drawn around each site. The percent max, average, and the volume of the ROI are recorded and the percent retention is calculated relative to a positive control on the left of the image which is equivalent to 100%.

**Figure 3.** A: Retention rate of delivered cells in myocardium after transendocardial, transepicardial, and intracoronary delivery in each animal. In healthy swine, approximately 3 times more cells were retained after transendocardial intramyocardial injections (~18%) compared to transepicardial intramyocardial injections (~6%). Intracoronary artery infusion led to retention rates of ~1%, lower than both transendocardial and transepicardial intramyocardial delivery. B: Average combined retention rate after transendocardial, transepicardial, and intracoronary cell delivery into myocardium. Data are given as the mean ± standard deviation. Helix transendocardial delivery resulted in 17.9 ± 3.1% retention in the beating heart of swine. Transepicardial delivery resulted in 6.0 ± 1.5% retention, while intracoronary artery infusion resulted in 1.0 ± 0.8% retention. P values indicate a difference between the 2 groups.
very difficult. While the percutaneous TE injection can be performed less invasively than surgical IM injection, this method has been considered a complex procedure because of the need for specialized catheter(s) and imaging modalities such as electromechanical mapping to deliver cells into target areas. Another hypothesized limitation of current IM injections whether transepicardially or transendocardially is the considerable amount of back leakage of injected stem cells during cardiac contraction through the holes made by the short straight needle. The Helix may overcome this limitation as its helical needle creates a long and spiral passage which self-seals in cardiac muscle contraction and upon needle removal. Moreover, Kumar, et al have demonstrated percutaneous TE cell delivery using this system under only fluoroscopic guidance to be accurate and safe.

This is the first study to evaluate stem cell delivery efficacy using the Helix transendocardial delivery system compared with other frequently used delivery methods of IC infusion and direct TP injection. In this study, significantly more cells were retained after TE injection than either TP injection with a straight needle or IC infusion. Some injected cells disappeared via venous and lymphatic drainage. Generally, most cells delivered by an IV or IC route are drained via the venous system and trapped in the lungs, liver, and other organs. A previous study using TE injection of radiolabeled agent has shown that despite direct IM delivery, significant amounts of radioactivity were found in non-targeted organs such as liver, kidneys, and lungs, but the concentration of radioactivity at the non-targeted organs was much lower than at the injection site. In this study, only some fractions of the delivered cells via TE and TP routes were found in the cardiac lymph node, while no signals were found in the IC group. There are limited safety data about the lymphatic engraftment of cells, thus future studies need to closely evaluate the fate and safety of the engrafted cells.

Limitations of this study should be noted. First, this animal study was performed using a healthy swine model and may not fully represent the clinical situation of myocardial infarction in humans. To more closely reflect future clinical needs in humans, an ischemic swine heart model may be preferred. Assessing the safety of injections and cell retention after inducing ischemia and/or infarction in a swine model is a future direction for investigation. Second, we only investigated the hyperacute retention of the cells because the study was designed to primarily assess the technical feasibility of delivering and imaging the \(^{18}\)F-FDG labeled cells delivered via the 3 main routes. Thus, our results refer to this specific point in time because it only focused on the efficacy of cell delivery. Future studies need to evaluate cell retention and distribution over time. Third, although some signal distributions were seen in non-cardiac organs via whole body PET, there was no detailed \textit{in vivo} or \textit{ex vivo} biodistribution assessment of the injected cells. Finally, there were no postmortem immunohistochemical analyses showing the viability quantitation of the delivered cells, although one of the shortcomings in tracking cells via \(^{18}\)F-FDG labeling is that the labeled \(^{18}\)F-FDG may be released into the body after cell death.

Conclusions: Our study indicated that the efficacy of cell delivery using a transendocardial helical infusion delivery system was more efficient than either transepicardial injection or intracoronary infusion. The Helix transendocardial delivery system has the potential to improve local cell delivery and retention in cardiovascular cell based therapy, thus potentially improving clinical outcomes. Further studies are needed to characterize retention over time and quantify efficiency in an ischemic swine heart model and humans.

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DISCLOSURES

Dr. Rouy is a former employee of BioCardia Inc. Drs. Altmann and Wong Po Foo are employees of BioCardia Inc. Dr. Stertzer is a director of BioCardia Inc. The other authors have nothing to disclose.

REFERENCES

6. Strauer BE, Steinhoff G. 10 years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: from the methodological origin to clinical practice. J Am Coll Cardiol 2011; 58: 1095-104. (Review)