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A New Coronary Retroinfusion Technique in the Rat Infarct Model: Transjugular Cardiac Vein Catheterization

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Abstract: Cell delivery via the retrograde coronary route boasts less vessel embolism, myocardial injury, and arrhythmogenicity when compared with those via antegrade coronary administration or myocardial injection. However, conventional insertion into the coronary sinus and consequent bleeding complication prevent its application in small animals. To overcome the complication of bleeding, we described a modified coronary retroinfusion technique via the jugular vein route in rats with myocardial infarction (MI). A flexible wire with a bent end was inserted into the left internal jugular vein and advanced slowly along the left superior vena cava. Under direct vision, the wire was run into the left cardiac vein by rotating the wire and changing the position of its tip. A fine tube was then advanced along the wire to the left cardiac vein. This modified technique showed less lethal hemorrhage than the conventional technique. Retroinfusion via transjugular catheter enabled efficient fluid or cell dissemination to the majority areas of the free wall of the left ventricle, covering the infarcted anterior wall. In conclusion, transjugular cardiac vein catheterization may make retrocoronary infusion a more safe and practical route for delivering cell, drug, and gene therapy into the infarcted myocardium of rats.

Key words: animal model, cardiac vein, cell transplantation, myocardial infarction, rats, transjugular

Introduction

Cell therapy has emerged as a promising approach to the regeneration of damaged cardiac tissue after acute myocardial infarction (AMI) and heart failure, and systematic reviews suggest improvement in global heart function [5]. The most popular method for cell delivery into the heart is through the intracoronary route, which features less arrhythmia occurrence and less myocardial injury when compared with the intramyocardial route [9]. However, antegrade intracoronary infusion has a limited ability to deliver cells to ischemic areas and a risk of coronary microembolism. Migration of inflammatory cells into the myocardial interstitium is known to take place at postcapillary venules, rather than at capillaries or arterioles [14]. So, retrograde intracoronary delivery can overcome these disadvantages and provide efficient cell dissemination within the myocardium, which allows a unique access to the ischemic myocardium [6–9, 15–17]. Recently, a clinical study [17] reported that retrograde intracoronary bone marrow cell implantation results in improvement of cardiac function in patients with chronic refractory angina. Retrograde coronary administration is considered to be an alternative method of delivery for cell therapy with the ability to safely deliver large number of cells regardless of oc-

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cluded or diffusely stenotic coronary arteries.

Rat models have been commonly used in studies of stem cell transplantation in the context of AMI [4]. However,there has often been difficulty in applying the promising method of retrograde coronary delivery in small-animal models. While retrograde coronary delivery commonly adopts catheter-based retroinfusion technique in large animals [13, 18, 19] and human beings [17], the direct insertion of a catheter into the thin left cardiac vein via the left superior vena cava or coronary sinus is needed in rats [15, 16]. Although a purse-string suture is used, the bleeding from the insertion site remains a big obstacle, as the wall of the vein is thin and fragile.

To overcome the complication of bleeding, we developed a modified retrograde intracoronary infusion technique via the jugular vein route in rats with myocardial infarction. The safety and feasibility of this technique for cell delivery was investigated.

Materials and Methods

Experimental protocol

Animals were purchased from the Shanghai Animal Administration Center. All animal experiment protocols were approved by the Animal Care and Use Committee of Fudan University and were in compliance with Guidelines for the Care and Use of Laboratory Animals, as published by the National Academies Press (NIH Publication No. 85–23, revised 1996).

A total of 40 adult Sprague-Dawley rats (200–250 g) were used in this experiment. All rats underwent open-chest ligation of the left anterior descending coronary artery (LAD) to develop the AMI model. Following LAD ligation, two animals underwent dissection to observe the anatomic path from the left internal jugular vein (LIJV) to left cardiac vein, and then underwent triphenyltetrazolium chloride (TTC) staining to confirm AMI. Eight animals underwent a conventional retrograde intracoronary technique as described previously (briefly, direct insertion of a catheter into the left cardiac vein via the coronary sinus) [15, 16]. Thirty animals were subjected to a modified technique (transjugular cardiac vein catheterization), followed by retrograde intracoronary infusion of Evans blue dye, stem cells, or PBS solution to confirm the perfusion area.

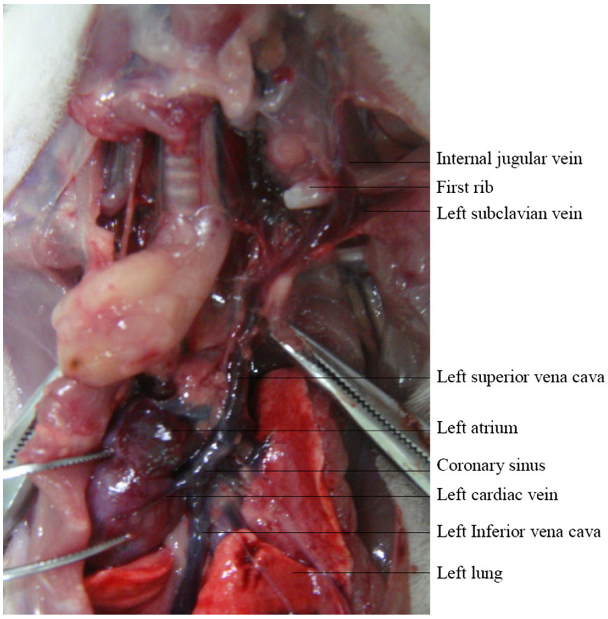

Fig. 1. Anatomy of the interventional path from the left internal jugular vein to left cardiac vein of rats.

Anatomic path from the left internal jugular vein to left cardiac vein of rats

The anatomic path from the LIJV to the left cardiac vein of rats was studied on autopsy [2, 12]. The LIJV runs down the side of the neck in a vertical direction, and at the root of the neck, it runs over the first rib and unites with the subclavian vein to form left superior vena cava. The left superior vena cava runs down and unites with the left inferior vena cava to form the coronary sinus. The left cardiac vein, which drains the left ventricular free wall, opens into the coronary sinus directly at an acute angle. This angulation can be stretched by pushing the heart downward and rightward (Fig. 1).

Transjugular cardiac vein catheterization in the rat with AMI

An experimental anterior-wall MI was induced as previously described by Antonio *et al.* [1]. Briefly, the rats were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), and ventilated using a rodent ventilator with room air at 80 breaths per minute. The heart was exteriorized through a left thoracotomy, and the LAD was occluded with a 6–0 polypropylene suture. Following the coronary occlusion, the heart was repositioned. Successful AMI was confirmed by TTC staining and the gross appearance of a color change from pink to dark red, along with hypokinesis. TTC staining

was performed as previously described [22]. Briefly, hearts were cut into transverse slices and incubated in a 1% solution of TTC in phosphate buffer for 5 min at 37°C, pH 7.4.

Half an hour after LAD ligation, transjugular cardiac vein catheterization was induced under additional anaesthesia with ketamine (30 mg/kg, i.p.). A 1 cm ventral cervical skin incision was made left of the midline with its caudal terminus at the level of the clavicle. Underlying salivary and lymphatic tissues was separated by means of blunt dissection to visualize the LIJV. Five millimeters of vessel cranial to the site where the LIJV passes under the clavicle was mobilized. After intravenous infusion of heparin (100 IU/kg), a 0.014 inch hydrophilic Fielder FC coronary guide wire (Asahi Intecc, Aichi, Japan) was properly shaped with a bent end, and then inserted into the LIJV. A temporal snare suture was placed at the end of LIJV to control bleeding from the insertion site. The wire was rotated and advanced slowly along the left superior vena cava. Under direct vision through the thin walls of the vein, the bent tip of the wire was run into the left cardiac vein by rotating the wire and changing the position of its tip, while the heart was pushed downward and rightward to stretch the angle between the vena cava and the left cardiac vein. A fine and flexible polytetrafluoroethylene tube catheter (0.35 mm ID and 0.6 mm OD, Shanghai Poly-fluorine Hardware Co., Ltd., Shanghai, China) was then advanced along the wire to the proximal-mid segment of the left cardiac vein. Selective catheterization of the desired cardiac vein was established, by which solution could be injected directly into the left cardiac vein. A temporal snare suture was placed at the distal left cardiac vein to prevent flush back of injected solution into the coronary sinus or the left vena cava.

Infusion of dye into AMI hearts by transjugular cardiac vein catheterization

Through the catheter, 1 ml of 0.4% Evans blue solution was injected slowly and selectively into the left cardiac vein with the snare tied. Five minutes after this, the snare was released to allow reperfusion, and the catheter was removed. The hearts were then removed after 5 min of observation and cut into five segments parallel to the apex-base axis.

Mesenchymal stem cells (MSCs) culture, labeling and infusion into AMI hearts by transjugular cardiac vein infusion

Rat bone marrow MSCs were isolated and cultured as described previously [11, 21]. A stock solution of poly-L-lysine (PLL, 0.15 mg/ml) was added to the culture media and mixed with superparamagnetic iron oxide nanoparticles (SPIO, Schering, Berlin, Germany, 100 mg/ml) for 60 min at room temperature on a rotating shaker. The culture media containing the SPIO-PLL complex were added to the cells such that the final concentration of iron was 50 mg/ml and the final concentration of PLL was 0.15 mg/ml [11]. The SPIO-MSCs were washed three times with phosphate-buffered saline (PBS), trypsinized with 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA; Gibco-Invitrogen, CA, USA). The SPIO-MSCs were then incubated with 1 μmol/l 1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide (DiR) (ABD Bioquest Inc., Sunnyvale, CA, USA) for 20 min at 37°C according to the DiR protocol.

After LAD ligation, 1×10^6 SPIO-Dir-MSCs resuspended in 1 ml PBS were retrogradely infused into the left cardiac vein with 5-min temporal occlusion using the method described (cell group, n=9). The PBS group (n=8) received PBS infusion after LAD ligation. The catheter was then removed, and the left jugular vein was tied. Surgical wounds were repaired, and the rats were extubated and returned to cages to recover.

To investigate the distribution of MSCs implanted, hearts were excised 24 h after cell infusion, and fluorescence imaging was performed by IVIS system (In-Vivo MS FX PRO, Carestream Health, Inc., Rochester, NY, USA) under 748 nm of excitation and 780 nm of emission. Then hearts were subjected to Prussian blue staining, which indicates the presence of iron. Briefly, pathological specimens were incubated for 30 min with 2% potassium ferrocyanide (Perl reagent) in 6% hydrochloric acid, and counterstained with nuclear fast red.

Statistical analysis

Descriptive variables were presented as mean ± standard deviation (SD) for measurement variables and percentages for numeration variables. Comparison between groups was performed with independent samples *t* test for measurement data, and Fisher's Exact Test for numeration data. Value of $P<0.05$ was considered statistically significant.

Table 1. Operative mortality and duration

	Conventional technique	Modified technique	*P* value
Death within 30 min after coronary ligation	12.5% (1/8)	10.0% (3/30)	<0.001
Death during tubing	42.9% (3/7)	0% (0/20)	<0.001
Tubing duration (min)	11.3 ± 3.9	15.6 ± 4.2	0.021

Results

Operative mortality

Among 8 animals that underwent the conventional technique, 1 rat died at 2 min following LAD ligation due to cardiac arrest, and 3 rats died during insertion of a tube into the left cardiac vein via the coronary sinus due to massive or uncontrolled hemorrhage. Among 30 animals that underwent the modified technique, 3 rats died within 10 min following LAD ligation due to cardiac arrest. However, the transjugular cardiac vein catheterization and subsequent retrograde intracoronary infusion of MSCs or PBS, were well tolerated, with no persistent arrhythmias or hemorrhage. As shown in Table 1, the tubing time of the conventional technique was a little shorter than the catheterization time of the modified technique (11.3 ± 3.9 min vs. 15.6 ± 4.2 min, P=0.021), however, the conventional technique showed greater lethal hemorrhage (3/7, 42.9%) than the modified technique (0/27, 0%), suggesting the safety of transjugular cardiac vein catheterization.

Within 24 h after solution infusion with the modified technique, 4 of 12 (33%) and 3 of 12 (25%) rats were lost in the PBS and cell groups, respectively. Apart from 3 rats that underwent dye infusion, 17 rat hearts (PBS group n=8; cell group n=9) were subjected to fluorescence imaging and Prussian blue staining.

Perfusion area of dye injection via the transjugular cardiac vein in AMI rat

TTC staining showed a staining defect in the anterior wall of the left ventricle after ligation of the LAD (Fig. 2A), suggesting the successful development of AMI. After infusion of Evans blue solution via transjugular catheter, The staining clearly extended throughout the layers of the left ventricular free wall. In contrast, the interventricular septum and the right ventricular free wall were spared from staining (Fig. 2B). The staining size, expressed as a percentage of LV myocardial area and measured in parasternal short axis views, averaged 55.6 ± 5.4%. Although the perfusion area via the left cardiac vein was larger than the infarct area by LAD occlusion, it covered the infarct area of the anterior wall.

Cell distribution via transjugular cardiac vein injection

Fluorescence imaging of extracted hearts revealed more fluorescent signal in the cell group than in the PBS group (P=0.001) (Fig. 3A, B). In hearts that received cell transplantation, cells containing Prussian blue-positive particles were sparsely distributed in the left ventricular free wall, while no blue-stained cells were detected in the interventricular septum and the right ventricular free wall (Fig. 3C). The distribution of blue-stained cells was consistent with the perfusion area of dye injection.

Discussion

The rat is an established experimental animal model for cardiac research, such as stem cell transplantation for myocardial infarction. Recently, retrograde intracoronary delivery has emerged as a promising route for cell therapy in both large animal experiment and clinical settings. However, the lethal bleeding of conventional insertion of a catheter into the coronary sinus encumbered its application in small animals. Drawing on the experience with the catheter-based percutaneous coronary interventional technique, we introduced a modified retrograde intracoronary infusion technique via the jugular vein route in MI rats.

Safety and ease of introducing a catheter into the cardiac vein of a rat is the principal advantage of the new technique described. In our study, 42.9% (3/7) of rats died from lethal hemorrhage during the conventional procedures of insertion into the coronary sinus and purse-string suture. In contrast, no animals (0/27) died during the modified procedure of transjugular cardiac vein catheterization. With the method described herein, the insertion of a catheter does not require puncture of the coronary sinus, and the tubing into the internal jugular vein has widely been used in rat experiments for decades.

To ensure successful transjugular cardiac vein cath-

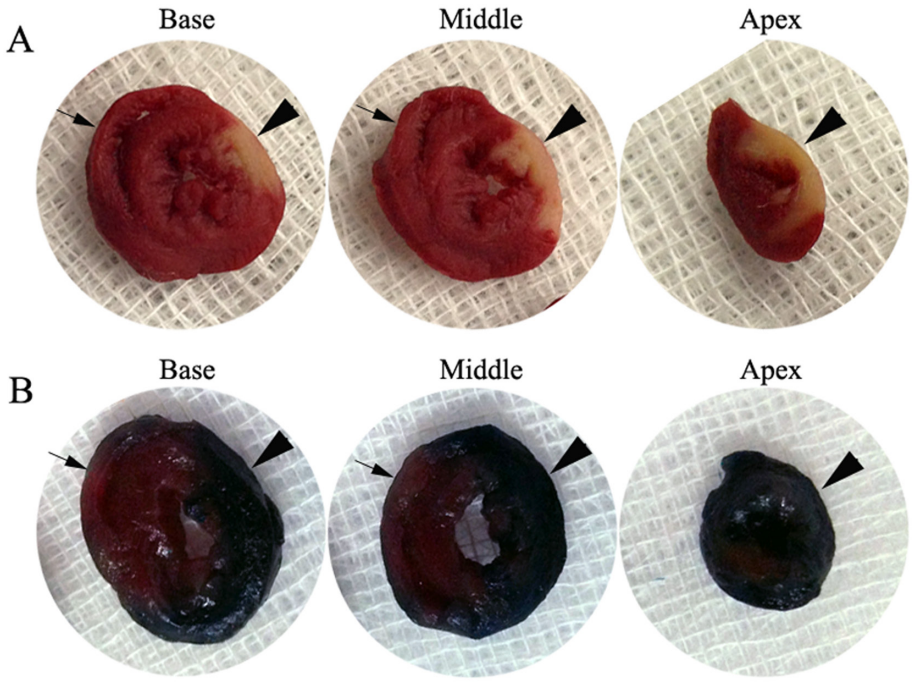

Fig. 2. Perfusion area by transjugular retrograde coronary infusion of Evans blue in LAD-ligated hearts. After ligation of the left anterior descending coronary artery, triphenyltetrazolium chloride staining showed a staining defect in the anterior wall of the left ventricle (A). The retrogradely infused Evans blue dye was mainly distributed at the left ventricular free wall (B), covering the infarcted anterior wall. Arrowheads denote the anterior wall of the left ventricle. Arrows denote the free wall of the right ventricle.

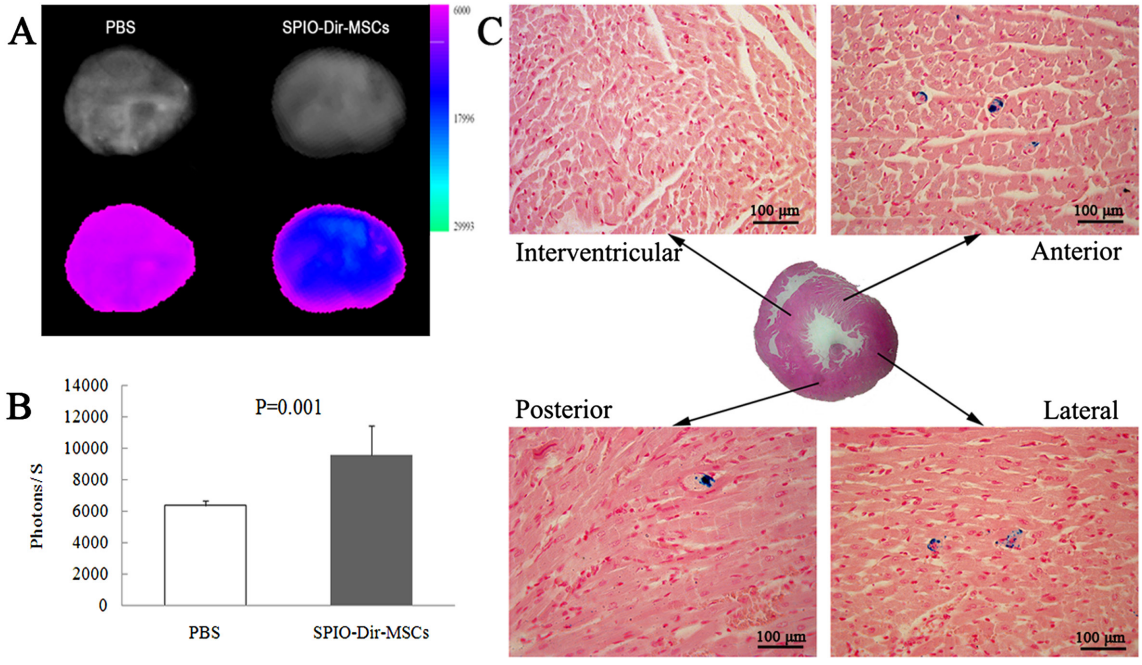

Fig. 3. Distribution of cells by transjugular retrograde coronary infusion. Fluorescent signals of rat hearts 24 h after cell/PBS injection is more intensive in the cell group than in the PBS group (A, B). Prussian blue staining demonstrates transplanted cells distributed in the anterior, lateral, and posterior walls of the left ventricle, while no blue-stained cells were detected in the interventricular septum (C).

eterization, attention should be paid to the following points. Firstly, the LIJV is the superior interventional access because the left path from the LIJV to the left cardiac vein of a rat is more linear than the right one. Furthermore, the internal jugular vein is especially prone to vessel spasm, so it should be gentle in manner to separate its neighboring soft tissues. Secondly, wire selection and shaping of the wire tip curve are basic considerations. The first line wire is 0.014 inch floppy wire, such as BMW (Abbott Vascular, Abbott Park, IL, USA) and CHOICE PT (Boston Scientific, Natick, MA, USA), as a stiffer wire carries the risk of penetrating the wall of the vein. Shaping of a wire tip curve is based on the vein anatomy. The length of wire tip bent should be equal to the diameter of the left superior vena cava, and the angle of the bent depends on the angle between the left vena cava and the left cardiac vein. Thirdly, advancing a guide wire into the cardiac vein is the key step. Although the left vena cava has an acute angle to the left cardiac vein in a rat, a wire tip angle of 30−45° of is usually enough because the angle can be increased by pushing the heart downward and rightward. During wire manipulation, the wire tip is advanced in short length (1−5 mm) and changes in the direction of wire tip are done simultaneously to select the left cardiac vein.

We found that the dye and cells infused via the left cardiac vein were located in the majority of areas of the free wall of the left ventricle, which covered the myocardium involved in the LAD related MI. Our data confirmed that the retrograde cells can reach the LAD-related infarct area, which is compatible with earlier studies [6, 15, 16]. These data suggested that transjugular retrograde coronary infusion may be useful as a route for cellular therapy of LAD-related MI, although the inconsistency in territory between the arterial branches and the venous branches made the retrograde method fail to deliver cells/drugs to the right coronary territory [20]. Maintenance and engraftment of transplanted cells in the target area are prerequisites for cell therapy. Based on this novel delivery method, we thought to adopt a spatially-focused magnetic targeting system we developed recently [10], to maximize the migration and homing of transplanted cells in order to optimize the efficacy of cell-based therapies using this small-animal model.

This small-animal model would be useful not only for investigating the feasibility of retrograde intracoronary infusion of various cell types including induced pluripotent stem cells, but also for investigating the efficacy of retrograde administration of cardioplegic solution, peptide, and vectors for gene therapy into the myocardium. More importantly, repeated retrocoronary infusion of stem cells or gene vectors could be achieved through a chronic indwelling cardiac vein catheter via the jugular vein of the rat using this novel technique. In addition, blood samples could be obtained through this catheter to calculate the coronary arteriovenous difference in concentration of various substances to study pathophysiological changes of heart diseases in small-animal models [3].

In conclusion, we introduced a transjugular cardiac vein catheterization technique in the MI rat and confirmed the perfusion area by using administration of dye and cells. It may make retrograde intracoronary infusion a more safe and practical route for delivering cell, drug, and gene therapies into the myocardium using rat models.

Acknowledgments

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