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Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary Venous Delivery

Implications for Current Clinical Trials

Dongming Hou, MD, PhD; Eyas Al-Shaykh Youssef, MD; Todd J. Brinton, MD; Ping Zhang, MD; Pamela Rogers, LATG; Erik T. Price, MD; Alan C. Yeung, MD; Brian H. Johnstone, PhD; Paul G. Yock, MD; Keith L. March, MD, PhD

Background—Several clinical studies are evaluating the therapeutic potential of delivery of various progenitor cells for treatment of injured hearts. However, the actual fate of delivered cells has not been thoroughly assessed for any cell type. We evaluated the short-term fate of peripheral blood mononuclear cells (PBMNCs) after intramyocardial (IM), intracoronary (IC), and interstitial retrograde coronary venous (IRV) delivery in an ischemic swine model.

Methods and Results—Myocardial ischemia was created by 45 minutes of balloon occlusion of the left anterior descending coronary artery. Six days later, 10^7 ^{111}In -oxine-labeled human PBMNCs were delivered by IC (n=5), IM (n=6), or IRV (n=5) injection. The distribution of injected cells was assessed by γ -emission counting of harvested organs. For each delivery method, a significant fraction of delivered cells exited the heart into the pulmonary circulation, with $26\pm 3\%$ (IM), $47\pm 1\%$ (IC), and $43\pm 3\%$ (IRV) of cells found localized in the lungs. Within the myocardium, significantly more cells were retained after IM injection ($11\pm 3\%$) compared with IC ($2.6\pm 0.3\%$) ($P<0.05$) delivery. IRV delivery efficiency ($3.2\pm 1\%$) trended lower than IM infusion for PBMNCs, but this difference did not reach significance. The IM technique displayed the greatest variability in delivery efficiency by comparison with the other techniques.

Conclusions—The majority of delivered cells is not retained in the heart for each delivery modality. The clinical implications of these findings are potentially significant, because cells with proangiogenic or other therapeutic effects could conceivably have effects in other organs to which they are not primarily targeted but to which they are distributed. Also, we found that although IM injection was more efficient, it was less consistent in the delivery of PBMNCs compared with IC and IRV techniques. (*Circulation*. 2005;112[suppl I]:I-150–I-156.)

Key Words: cells ■ ischemia ■ myocardial infarction ■ catheters

Despite recent advances in the treatment of cardiovascular diseases, heart disease continues to be a major cause of morbidity and mortality worldwide.¹ The last several years have witnessed the emergence of several cell types as potential therapeutic modalities for treating both coronary artery disease and congestive heart failure.^{2–4} A range of cells are being tested for enhancement of angiogenesis^{5–7} and modulation of myocardial remodeling after acute infarction.^{8,9} Moreover, there have been attempts to evaluate the effects of cells to repair damaged myocardium in the context of cardiomyopathy.^{10–12}

Several techniques have been used to deliver therapeutic progenitor cells or genes to the heart. In humans, 2 delivery modalities have been used in pilot clinical trials: intracoro-

nary (IC) infusion^{8,9,13–17} and intramyocardial (IM) delivery, conducted either by direct IM injection during open-chest surgery^{18,19} or percutaneously by various endomyocardial injection techniques.^{20,21} Two additional delivery modalities have been evaluated in animal models and may have promising clinical applications in the future; these techniques include infusion into the coronary venous system^{22–26} and the pericardial space,^{27–30} respectively, as conduits to deliver a variety of agents to the heart.

Although several animal as well as human trials have suggested potential benefits of cells derived from peripheral blood, bone marrow, or mesenchymal tissues in promoting angiogenesis and myocardial repair, the actual fate of locally delivered cells has not been thoroughly assessed; and no

From the Indiana University School of Medicine (D.H., E.A.-S.Y., P.Z., P.R., B.H.J., K.L.M.), the Krannert Institute of Cardiology (D.H., E.A.-S.Y., B.H.J., K.L.M.), and the Richard L. Roudebush VA Medical Center (K.L.M.), Indianapolis, Ind, and Stanford University School of Medicine (T.J.B., E.T.P., A.C.Y., P.G.Y.), Stanford, Calif.

The first 2 authors contributed equally to this work.

Correspondence to Keith L. March, MD, PhD, Indiana Center for Vascular Biology and Medicine, 975 W Walnut St, IB 441, Indianapolis, IN 46202. E-mail kmarch@iupui.edu

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study has directly compared various local-delivery methods with respect to cell distribution. Localized delivery of cells to targeted organs is expected to be helpful in maximizing local benefit while minimizing the potential harmful effects^{31–34} that these cells may exert on other nontargeted organs. In this study, we evaluated the distribution of peripheral blood mononuclear cells (PBMNCs) after IM, IC, and interstitial retrograde venous (IRV) delivery in an ischemic swine model.

Methods

Animal handling and care followed the recommendations of the National Institutes of Health (NIH) guide for the care and use of laboratory animals. All protocols were approved by the animal care and use committee at Indiana University School of Medicine.

Sixteen Yorkshire domestic pigs (25 to 35 kg) of either sex were included in the study. Myocardial infarction was created in each animal by inflation of an occlusive angioplasty balloon in the proximal left anterior descending coronary artery (LAD). Six days after ischemia, study animals were randomly assigned to 1 of 3 groups: IC delivery (n=5), IM delivery (n=6), or IRV delivery (n=5). Animals in the IC and IM groups received 10^7 ¹¹¹In-oxine-labeled human PBMNCs. Within the IRV group, 5 animals received 10^7 ¹¹¹In-labeled human PBMNCs and 3 additional animals received 10^7 ¹¹¹In-labeled human adipose stem cells (ASCs). Animals were evaluated for cell distribution by γ -counting of tissue samples and whole-body imaging 1 hour after cell delivery.

Animal Model

Study animals were sedated with intramuscular ketamine (20 mg/kg), xylazine (20 mg/kg), and telazol (45 mg/kg). After intubation, anesthesia was maintained with isoflurane (2.5%). Arterial access was obtained via the common carotid artery with a cutdown technique, and an 8F vascular sheath was used to cannulate the artery. Left heart catheterization was performed with a hockey stick catheter (Cordis) for coronary cannulation and a pigtail catheter (Cook) for ventriculography. A femoral artery sheath was placed to maintain continuous hemodynamic monitoring during the procedure. All study animals had a normal baseline coronary angiogram and left ventriculogram.

Myocardial ischemia was created by inflating an angioplasty balloon for 45 minutes in the proximal LAD immediately distal to the first septal perforator. All study animals received IV amiodarone to minimize ventricular arrhythmias; the amiodarone dosage was a 75-mg IV bolus over 10 minutes given just before balloon inflation, followed by a 1 mg/min infusion continued for the duration between balloon inflation and deflation (45 minutes). Nevertheless, sustained ventricular tachycardia and ventricular fibrillation occurred during balloon inflation in every animal (2.9 ± 1.6 episodes per procedure) and were terminated with biphasic DC cardioversion (Medtronic). Mean arterial pressure and left ventricular end-diastolic pressure were measured at baseline, 1 hour after myocardial infarction creation, and immediately before cell delivery.

The extent of myocardial damage created by coronary occlusion was assessed by 2 methods: ejection fraction (EF) and tetrazolium trichloride (TTC) staining of harvested hearts. Left ventriculography was performed at baseline and just before cell delivery, and EF was calculated for each acquired image on a Phillips BV Pulsera workstation and left ventricular analysis Inturis for Cardiology software, release 2.1. TTC staining was performed according to a standard protocol,³⁵ during which hearts were removed immediately after the animal was humanely killed and were sliced into 5 sections of 1.5- to 2.0-cm thickness along the apical-basal axis. These sections were immersed into TTC solution for 10 minutes at 37°C. Afterward, myocardial ischemic areas (pale areas not staining with TTC solution) were quantified by manual tracing and NIH imaging software and were expressed as a percentage of the total left ventricular area.

Isolation and Labeling of PBMNCs

Fresh blood products were obtained from the human blood bank. PBMNCs were isolated by Ficoll density-gradient centrifugation³⁶ (Pharmacia Biotech) of the buffy coats. Remaining red blood cells were destroyed in lysis buffer, and the isolated cells were washed several times with phosphate-buffered saline solution.

The isolated cells were then labeled with ¹¹¹In as described.^{37,38} The labeling was done by incubating the cells (10^7) with ¹¹¹In (0.5 mCi in 0.5 mL of serum-free medium) for 30 minutes at room temperature. Unbound label was removed by washing the cells twice with serum-free Dulbecco's modified Eagle's medium. Labeling efficiency, defined as the fraction of label retained by the cells (vs the supernatant) after the incubation, was measured by γ -counting (Packard Auto-Gamma Cobra II) at $66 \pm 9\%$.

Delivery Methods

IRV Delivery

Venous access was obtained via the internal jugular vein. The coronary venous system was cannulated with a novel, investigational, double-balloon catheter (Venomatrix, Inc; Figure 1A). This device comprises 2 concentric catheters; a proximal, outer catheter bearing a large balloon (1.0×0.5 cm), and a distal, inner catheter bearing a smaller balloon (0.5×0.5 cm); the distal balloon is used to minimize washout of delivered cells into the systemic circulation via distal, low-resistance venovenous anastomoses.

First, the coronary sinus was engaged by the proximal catheter, followed by its advance into the proximal interventricular vein (AIV) over a 0.014-inch flexible guidewire. A coronary sinus venogram was performed initially to delineate the anatomy and provide a roadmap for subsequent cannulations. Subsequently, the distal catheter was introduced through the proximal device and advanced into the mid-to-distal AIV. Catheter placement was confirmed angiographically by injection of 1 to 2 mL of diluted nonionic contrast agent into the AIV, and proper positioning was determined by the presence of significant contrast blush in the area of the myocardium between the 2 balloons, marking interstitial access by the pressurized fluid. In the event that blush was not observed, the catheters were repositioned to another site and then retested. In studies performed in the first 5 animals, the distance between the proximal and distal balloons was set at 4 cm, and injection pressure was maintained at 150 ± 16 mm Hg, resulting in delivery over 8 ± 3 seconds. Cell counts were 10^7 in 10 mL of solution. After delivery, both balloons were deflated after 5 minutes to achieve maximum local delivery. Continuous hemodynamic monitoring was maintained during the whole procedure with a femoral arterial sheath.

IC Delivery

Five to 7 days after myocardial ischemia, coronary angiography was repeated to evaluate the site of previous balloon inflation, which typically showed minimal luminal irregularities (10% to 20%) without significant obstructive lesions. An angioplasty balloon of equivalent size to the artery was inflated at low pressure (2 atm) in the same location, and 10^7 ¹¹¹In-labeled cells (10 mL) were infused over 30 to 45 seconds. The angioplasty balloon was deflated after 3 minutes (Figure 1B).

IM Delivery

A median sternotomy was performed, and the pericardium was opened to expose the anterior surface of the heart. A 1-mL tuberculin syringe with a 27-gauge needle was used to inject 10^7 ¹¹¹In-labeled cells suspended in a total 2-mL volume at each of 10 evenly spaced sites in the anterior left ventricular wall between the diagonal branches of the LAD (200- μ L injection volume at each site; Figure 1C).

Tissue Harvesting and Measurement of Radioactivity

All animals were euthanized 1 hour after cell delivery by a lethal dose of pentobarbital (65 mg/kg). Distribution of radioactive cells was measured in each animal. Qualitative whole-body scans were performed with an Ecat Exact HR+PET scanner (63 planes, 15.5-cm

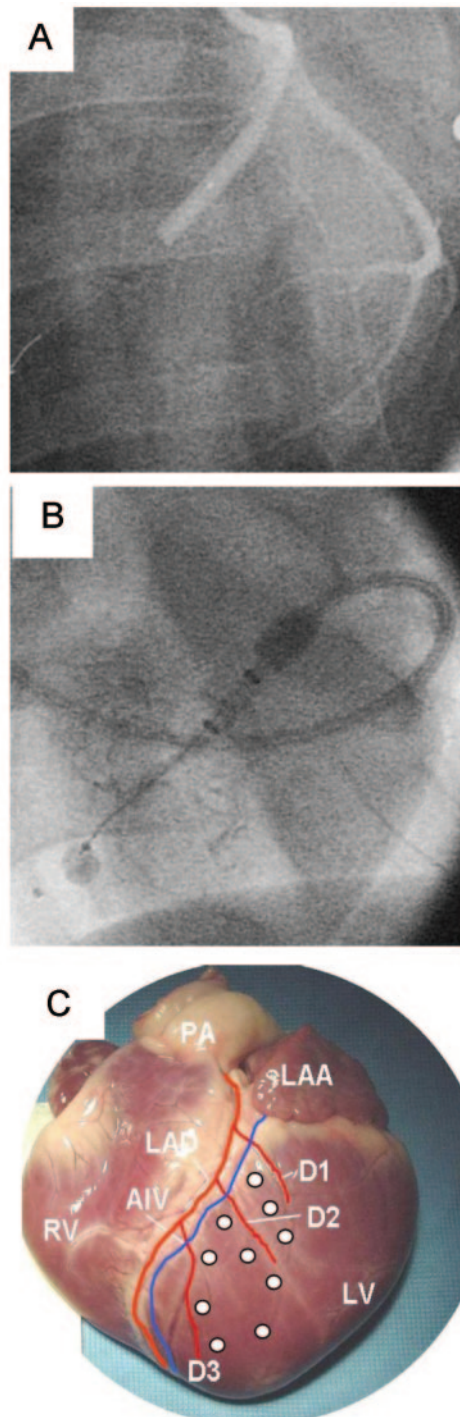


Figure 1. Position of the infusion sites for IC, IRV, and IM cell delivery. A, Angiogram of the left coronary artery after balloon occlusion of the LAD (middle). B, Dual-balloon occlusion of the AIV before delivery. The proximal balloon is clearly visible at the top right of the image. The radiopaque marks approximately delineate the delivery site. C, Pattern for IM injections between the diagonal branches of the left ventricle. Abbreviations are as defined in text.

field of view) system, which has a resolution of 4.2 mm. Radioactivity was quantified in tissue samples with a Packard Auto-Gamma Cobra II. All major visceral organs (heart, lungs, liver, spleen, and kidneys), as well as samples from skeletal muscle (tibialis anterior), skeleton, and circulating blood, were collected and weighed. The heart was transversely sliced into 5 sections of 1.5- to 2.0-cm

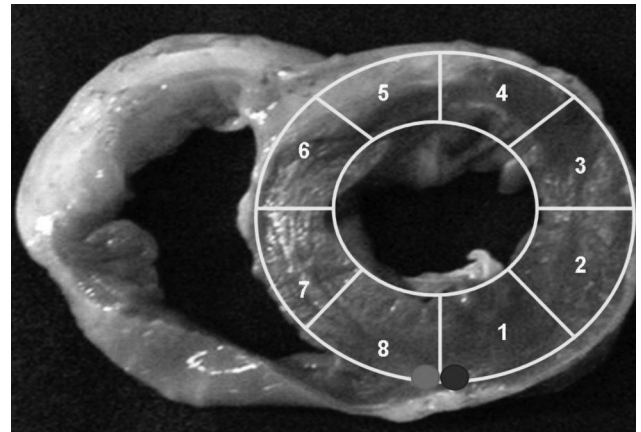


Figure 2. Typical cross section of the basal region of the heart, showing sectioning of the left ventricle for ^{111}In -labeled cell distribution analysis. Red and blue circles are the LAD and AIV, respectively. Abbreviations are as defined in text.

thickness along the apical-basal axis before being subdivided further into 8 wedge-shaped pieces of approximately equal dimensions (Figure 2). An ≈ 1 g piece of tissue from each of the remaining organs (1 from each lobe of the lung) was collected for determination of radioactive counts. The amount of radioactivity in the entire organ was determined from the measured whole-organ weights, except for blood, bone, and muscle, which were extrapolated on the basis of standard estimates for each, amounting to 5.5%, 17%, and 36%, respectively, of the mass of the entire animal. Radioactivity retained by the syringe and catheter after delivery to the pig was determined in 8 instances by quantifying emissions along the entire length of the devices.

Statistics

Results are presented as mean \pm SEM. Data were compared with a 1-way ANOVA and Tukey-Kramer multiple comparisons tests (GraphPad InStat software), with differences between data sets considered significant at $P < 0.05$.

Results

Ventricular arrhythmias occurred during each coronary balloon occlusion. Cardioversion was unsuccessful in 3 animals, and another 5 animals died suddenly 1 to 2 days after infarction (32% cumulative attrition rate). This infarction model was characterized by a myocardial ischemia volume of $26 \pm 5\%$ of the whole left ventricle (Figure 3A) and an EF decrease of 46%, from $65 \pm 8\%$ at baseline to $35 \pm 7\%$ after 6 days (Figure 3B), with a strong correlation between myocardial ischemic area and the drop in EF ($R^2 = 0.93$; Figure 3C). Hemodynamic parameters were also consistent in our model, with mean arterial pressure dropping from 60 ± 7 mm Hg at baseline to 46 ± 5 mm Hg after 6 days, and left ventricular end-diastolic pressure increasing from 9 ± 3 mm Hg at baseline to 14 ± 2 mm Hg after the same interval.

In 8 of 16 experimental pigs distributed among all groups, the delivery system (syringe/catheter) was exhaustively evaluated for retained radioactivity after cell delivery. Overall, an average of $12 \pm 3\%$ of labeled cells was retained in the delivery system after injection (data not shown).

Whole-body γ -scans demonstrated that a significant percentage of cells was entrapped in the lungs after each delivery technique (Figure 4). γ -Count values of tissues harvested 1 hour after delivery, multiplied in each case by the total weight

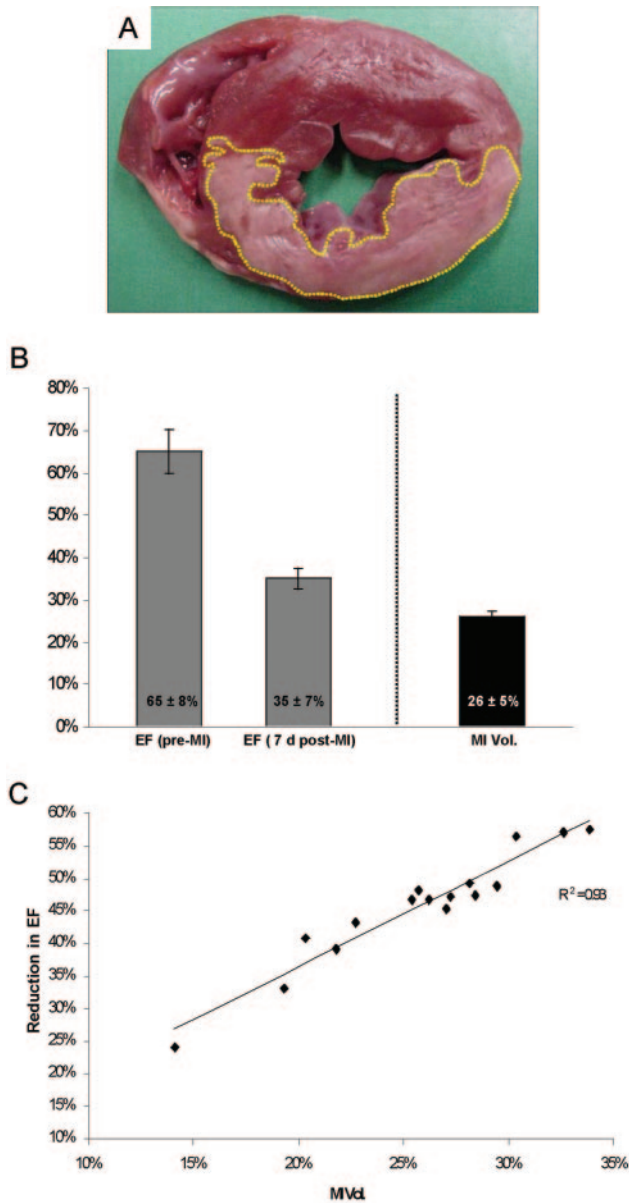


Figure 3. Extensive myocardial damage is evoked by coronary occlusion and reperfusion injury. A, Typical cross section of an infarcted porcine heart 1 week after ischemic insult. The infarcted tissue is delineated with TTC dye (pale area outlined by dotted yellow line). B, The volume of infarcted left ventricular myocardium (MI Vol) was determined from values for each heart, derived by multiplying the thickness of each section by the areas of infarction, which were determined by planimetry. The EF was determined for each heart immediately before (pre-MI) and at 7 days after (7 d post-MI) LAD occlusion. Average values (\pm SEM) were determined from 16 animals receiving PBMC + 3 animals receiving ASCs, which are part of an overlapping complementary study. C, The total MI area was plotted against the decrease in EF for each animal ($n=17$). Abbreviations are as defined in text.

of the respective organ, were used to determine efficiencies of retention for each visceral organ (defined as the ratio of the locally retained radioactivity to the total radioactivity placed in the delivery device). This computation revealed that $50\pm 5\%$ of delivered cells were distributed to the visceral organs. The remaining radioactivity was found to have been

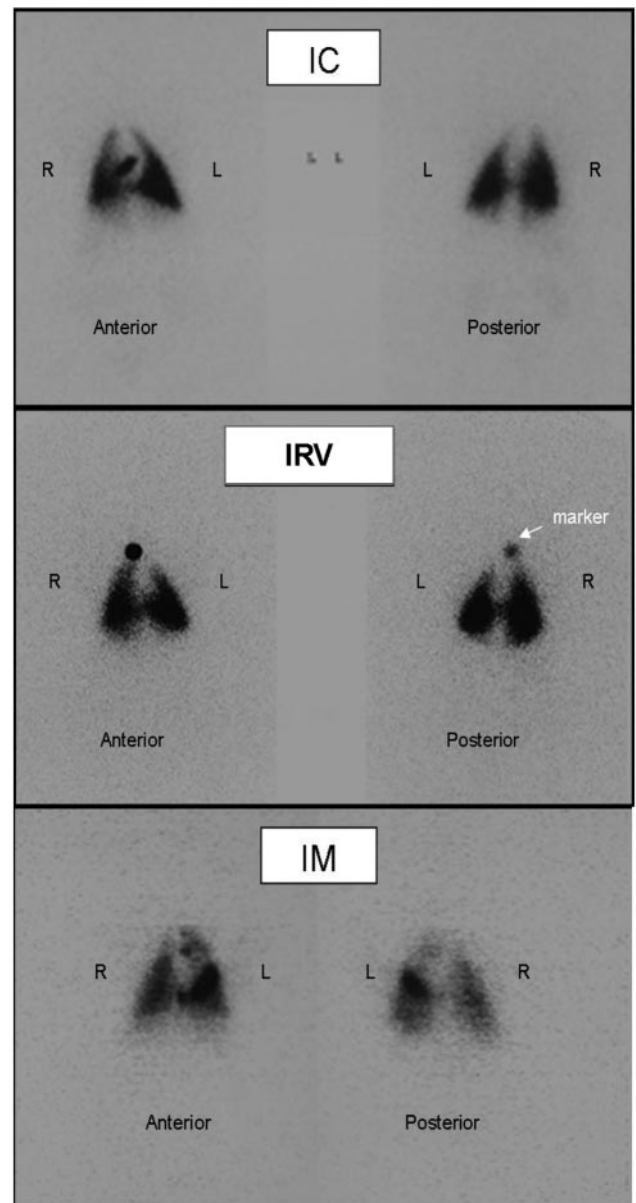


Figure 4. Whole-body γ -scans of pigs 1 hour after cell infusion by IC (top), IRV (center), and IM (bottom) routes. Each animal was analyzed in both supine (anterior) and prone (posterior) positions. A radiographically visible marker was used for orientation after IRV delivery. Abbreviations are as defined in text.

retained within the devices (as noted previously) or within the musculoskeletal system and circulating blood.

Further assessment of cell distribution to the visceral organs demonstrated that the organs receiving the greatest fraction of cells for all delivery modalities were the lungs, with $26\pm 3\%$ (IM), $47\pm 1\%$ (IC), and $43\pm 3\%$ (IRV) of cells localized to the lungs. Significantly lower percentages of cells were found within the remaining organs (Figure 5).

In an effort to further evaluate the geometry as well as efficiency of localized myocardial delivery, detailed quantitative and topographic analyses of cell delivery were evaluated. Significantly more cells were retained in the heart after IM injection, $11.3\pm 3\%$, in comparison with IC $2.6\pm 0.3\%$ ($P<0.05$). IRV infusion efficiency was lower than IM

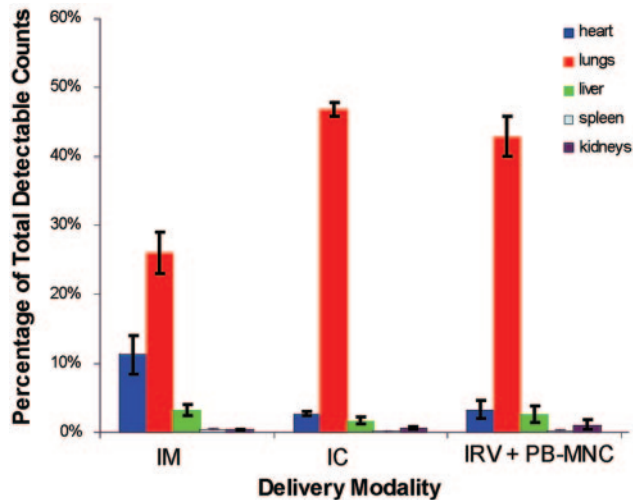


Figure 5. Distribution of radioactive counts in major organs after IM, IC, and IRV delivery of cells. The entire heart was sectioned and analyzed, whereas a small section only of all other organs was analyzed, and counts were normalized to organ weight. Abbreviations are as defined in text.

($3.2 \pm 1\%$), but this difference did not reach statistical significance (Figure 6). Delivery efficiency was least consistent in the IM group (18-fold variability among the animals, where variability was defined as the maximum delivery efficiency divided by the minimum efficiency within each delivery technique). The IM group was significantly more variable in comparison with the IC group (2-fold variability; *F* test for difference of variability, 0.00032). The IRV modality trended toward less variability (9-fold variability) than that of IM, but this difference was not significant by the *F* test (0.11). The topographic myocardial distribution of delivered cells was also evaluated. Cells delivered by the IRV route were distributed predominantly to the heart base, atria, and right ventricle, whereas cells were essentially localized to the anterolateral left ventricular wall in the IM modality and to the anterolateral and apical regions of the left ventricle, with some cells being distributed to the right ventricle after IC delivery (Figure 7).

The IRV delivery technique readily lends itself to modulation of delivery pressures as desired. Accordingly, in an

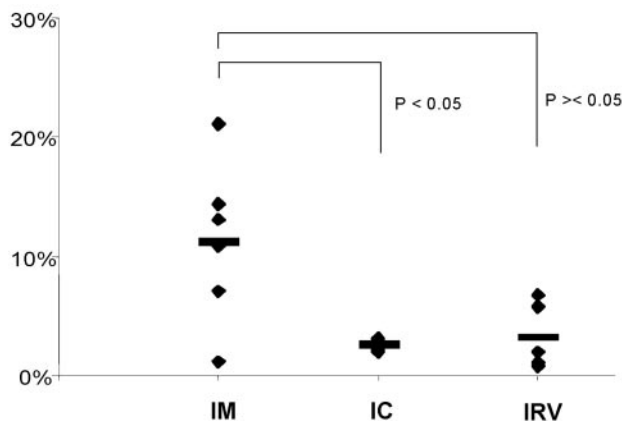


Figure 6. Variability of radiolabel retained for each delivery case by IM, IC, and IRV routes. Triangles represent counts retained in each heart. Vertical bars represent the average for each of the 3 modalities. Abbreviations are as defined in text.

effort to optimize delivery in a pilot group of 3 animals that received identical predelivery treatment, we tested the effect of modifying the IRV approach by defining a more restricted delivery region (2.5 cm long) as well as delivering a population of larger, mesenchymal stem cells, ie, ASCs, that have been identified as potentially capable of modulating survival of ischemic tissue. In these animals, the shorter interballoon distance resulted in substantially increased delivery resistance and was associated with an increase in the average injection pressure, to 300 mm Hg, to achieve delivery over an extended 13 ± 8 seconds. These deliveries yielded average myocardial retention values of $13 \pm 6\%$ and extramyocardial deliveries similar to all other delivery groups (data not shown). During all IRV procedures, it was noted that variability existed in the degree of venovenous drainage visible on contrast dye infusion under fluorography. The degree of runoff was highly predictive of the efficiency of cell retention (data not shown). In the majority of cases, drainage could be eliminated or greatly minimized by repositioning the catheter so that either of the 2 balloons occluded the conduit.

Discussion

In this study, we have demonstrated and characterized for the first time the substantial distribution of radiolabeled cells to visceral organs when performing localized myocardial delivery by any of 3 fundamental modalities. Indeed, we have demonstrated that most of the cells delivered by various techniques were distributed to extracardiac organs shortly after delivery. This widespread, nontarget distribution was evident for each of 2 disparate cell types: hematopoietic, represented by the PBMNCs, delivered by 3 approaches, and mesenchymal stem cells, represented by ASCs, delivered in a pilot group by an IRV approach. The observation of such a distribution highlights the need to monitor for unintended effects, particularly in the context of the potential proangiogenic effects of some delivered cells. This observation uncovers the potential utility for using techniques to track the distribution of delivered progenitor cells in human clinical trials. Moreover, long-term follow-up of treated patients may be important for evaluation of the possible effects of these cells on extracardiac organs.

One remarkable feature common to all 3 delivery modalities studied was the largely right-sided distribution of cells, marked by pulmonary cell trapping far in excess of that by filter organs (eg, liver and spleen) on the systemic/left-sided circulation. This finding strongly highlights that cell delivery by each of these approaches is accompanied by egress of cells from the myocardium largely into the myocardial venous or lymphatic drainage, rather than into arterial conduits or the ventricular lumen. The subsequent pulmonary trapping, beyond serving as a key indicator of right-sided egress from the cardiac target, may reflect mechanisms that include cell surface antigenicity and cell activation. The possibility that the human origin of the delivered cells or their activation by handling processes played roles in dictating pulmonary trapping cannot be ruled out, but it seems unlikely that such considerations would have artificially caused the observation of low-level cardiac cell extraction.

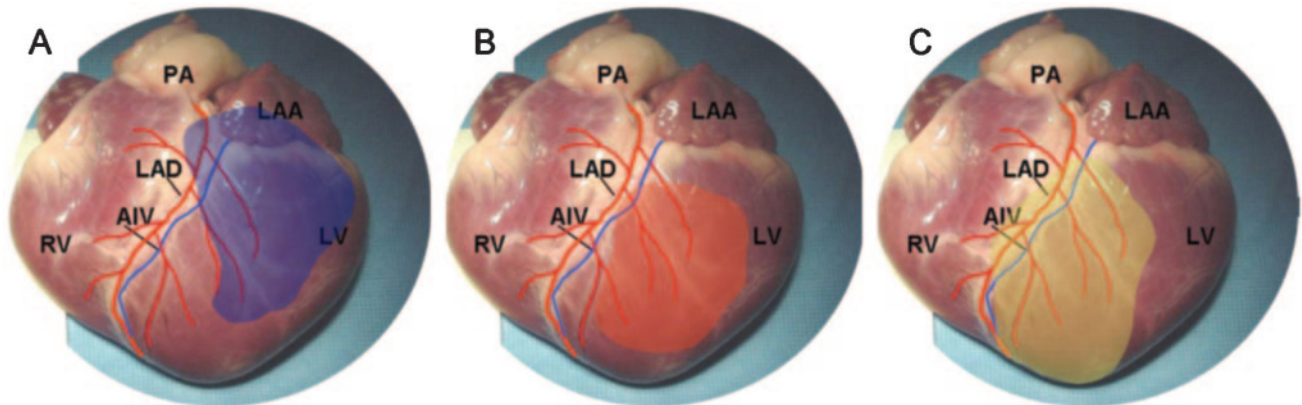


Figure 7. Representation of average distribution patterns in hearts receiving (A) IRV, (B) IC, and (C) IM cell deliveries. PA indicates pulmonary artery; RV, right ventricle; LV, left ventricle; AIV, and LAA, left atrium. Other abbreviations are as defined in text.

In this study, we also performed comparative analyses of myocardial delivery efficiency and consistency for 3 delivery modalities and modified the hydraulic parameters of delivery particularly relevant to 1 of them. Using PBMNCs, we demonstrated that IM delivery was somewhat more efficient but was characterized by variability in the locally delivered dose. Importantly, we are yet unable to identify the key predictors of delivery efficiency for IM injection. This finding suggests the potential importance of using quantifiable methodologies, such as the readily available nuclear labeling approaches used in this study, to track cells *in vivo* during any clinical cell-delivery study. Admixture of labeled cells would permit routine identification of those patients who received high locally retained cell doses for comparison with those manifesting low local retention of the cell dose, with respect to clinical outcomes and therapeutic responses. In addition, those subjects who receive lower localized myocardial delivery would conversely harbor the highest doses in the visceral organs, which may have implications for potential side effects.

In this study, we evaluated for the first time the use of a double-balloon catheter for IRV delivery. This novel device offers the potential advantage of increasing local delivery for the IRV technique by minimizing washout of delivered cells through distal venovenous communications, which have been described as a key anatomic source of variability for the IRV approach.²⁵ This delivery technique offers the unique potential to modulate the hydraulic properties of delivery by manipulation of injection pressure as a variable, thus modifying the extent of local capillary network disruption and affecting interstitial access. We accordingly evaluated the IRV route by using PBMNCs at an initially moderate retroinfusion pressure of 150 mm Hg. Subsequently, we performed a pilot study to evaluate the IRV route for delivering ASCs at higher retroinfusion pressures (300 mm Hg) that were achieved by decreasing the distance between the balloons demarcating the delivery compartment. The results together demonstrate the need for careful attention to delivery variables when comparing delivery techniques, as indeed, a set of changes in the IRV delivery paradigm substantially increases its efficiency. Accordingly, much more work will be required to disentangle the multiple variables that affect delivery efficiency and possibly efficacy.

Qualitative observations of the presence of venovenous collateral channels, evidenced by the behavior of the contrast agent administered before cell delivery, suggested that these channels play a key role in determining the variability of the delivery efficiency for this method, with relatively low delivery efficiency associated with a greater extent of venovenous collateral channels that would predict high washout and those with a high delivery efficiency (associated with a single AIV) with highly effective occlusion of large-caliber venous washout by the distal balloon. Such predictability distinguishes the IRV approach from the IM injection technique, for which the source of variability is not yet predictable. However, we hypothesize that this may be related to a variable degree of needle access into the venous networks draining the myocardium (data not shown). These findings support further investigation of the IRV technique with prospective and more quantitative evaluation of the effects of minimizing outflow via venous collaterals to enhance myocardial delivery efficiency. The use of coronary veins to deliver cells may provide an important alternative to IM injection to provide delivery in the context of occluded coronary arteries in patients who are not suitable candidates for IC delivery strategies. An additional attractive feature motivating study of the parameters of IRV delivery is the favorable clinical profile of venous access compared with arterial access.

The use of IC cell infusion continues to be a popular approach, given the familiarity of most cardiologists with this well-established procedure.^{9,13,14} This study demonstrates a superior degree of reproducibility for cell delivery into and around infarcted myocardium with this approach, but it also demonstrates the lowest average efficiency for myocardial delivery of PBMNCs in comparison with the other approaches tested. These findings clearly support the detailed study of extracardiac distribution of other cell types and also suggest the importance of tracking cells *in vivo* in the context of IC delivery in human trials.

One limitation of this study was the use of human-derived cells in a swine model (albeit acute model) focusing on cell disposition within 1 hour of delivery. Although characterization of the distribution of animal progenitor cells could form the basis for additional studies, an early preclinical understanding of the behavior of human cell types that are the active agents of ongoing or planned human trials seemed of

substantial initial interest. In addition, we recognize that although our findings of extracardiac distribution of delivered cells now call attention to a potentially significant topic in the clinical setting, further studies evaluating the ongoing fate of cells after longer durations of time will be necessary to extend these results and to understand in greater detail the outcomes of local cell delivery to the cardiovascular system.

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