Intracoronary Injection of Large Stem Cells
Size Matters

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Heart failure is a disease with an unmet need for effective treatments. Cardiac stem cell therapy has emerged as a promising approach to potentially replace scarred tissue within a failing heart. Delivery of various stem cells from different origins (eg, bone marrow, mesenchymal, endothelial, and cardiac progenitor) has been shown to improve cardiac function in animal models of ischemic injury; however, the efficacy of these cells in patients has been less convincing, despite multiple trials.1-4 One of the main barriers for effective stem cell treatment is the delivery and retention of the cells to the affected areas in the heart. Current cardiac-specific delivery methods include intramyocardial delivery, intracoronary injection, and pericardial delivery. Intramyocardial delivery can be performed either surgically or using percutaneous endocardial injection catheters.5 Pericardial delivery involves placement of pericardial stem cell sheets,6 which is typically performed surgically, but a percutaneous approach is also feasible.7 Intracoronary injection of cells can be accomplished using approaches that deliver cells via an antegrade intracoronary artery injection or a retrograde sinus injection. Among the various procedures, antegrade intracoronary artery injection is attractive because it is the least invasive and simplest approach; cells can be delivered using a 5F or 6F catheter using standard femoral or radial access. In addition, this approach can be integrated easily into clinical practice. Antegrade intracoronary injection is also used for delivering other therapeutic materials, such as vectors carrying genes8; however, there is one important aspect that needs to be addressed when injecting stem cells, that is, cell size.9,10 Optimal cell size is thought to be limited by the size of the coronary capillaries, which are thought to be ≈7 to 10 µm11; the size of some of the stem cells easily exceed this diameter. Therefore, microvascular plugging as a result of stem cell injection can theoretically occur and may cause microinfarction when the cells injected are too large for the diameter of the capillary bed.

In this issue of Circulation: Cardiovascular Interventions, Gallet et al12 report their findings from a preclinical study of intracoronary cardiospheres injection in a pig model of ischemic heart failure to test the hypothesis that cardiospheres delivered via this route were both safe and efficacious. Cardiospheres are self-assembling multicellular clusters derived from cardiac explants grown in culture. These cardiospheres are composed of heterogeneous cell types, including cardiac stem cells and multiple subpopulations.13 Previous studies relied on dissociation of the cardiospheres to smaller single cells, termed cardiosphere-derived cells (CDC) to avoid potential microvascular occlusion.13,14 By controlling the cell culture conditions, the authors developed an optimized protocol to harvest relatively uniform cardiospheres with a diameter of 45±23 µm. The authors showed that intracoronary injection of 50×10⁶ cardiospheres of this diameter does not cause elevation in serum cardiac enzymes in naïve pigs and used the same dose for treating pigs with myocardial infarction–induced heart failure. Acutely after cardiospheres injection, although the authors reported that one animal had impaired coronary flow compared with the preinjection angiogram, they concluded that this event was likely because of coronary spasm. They also reported no evidence of microinfarction as assessed by changes in serum cardiac enzymes. Cardiospheres delivered by intracoronary injection were also found to be efficacious and resulted in a decreased scar volume and increased myocardial perfusion and preservation of left ventricular ejection fraction at the chronic stages.

Although the results from this study are encouraging, many issues remain. Considering the size of the cardiospheres, the majority of them should have been entrapped in the capillaries leading to microvascular occlusion. If this is the case, it is not immediately obvious why microinfarctions were not detected after intracoronary cardiospheres injection. An obvious explanation is the sensitivity of the methodology used to detect microinfarctions, but there are also additional plausable explanations for the absence of detectable microinfarctions: (1) cardiospheres transmigrate across the endothelium in larger caliber vessels before being entrapped in the capillaries; (2) despite size differences, cardiospheres somehow pass through the capillaries, enter the systemic circulation, and take up residence in other organs; (3) cardiospheres cause transient microvascular occlusion, but transmigrate across the capillary endothelial cells at entrapped locations; and (4) cardiospheres remain entrapped in capillaries, but distal perfusion of the occluded capillary bed is maintained by collateral capillaries.

In the first scenario, the cells need to adhere to the coronary artery wall before reaching the capillaries and then transmigrate. Adhesion to the vessel wall and transmigration of cells across the endothelial barrier is likely one of the functions that differs between stem cell types. It is interesting that Gallet et al12 reported that larger cardiospheres (>150 µm) were more...
likely to be captured within the delivery catheter, suggesting that these larger cardiospheres might have enhanced adhesive function. This property may be advantageous in that it might help cardiospheres attach to the vessel wall and potentially migrate into the parenchyma or, in this case, the ischemic or infarcted myocardium.

In the second scenario, the cardiospheres probably change their shape or deform to facilitate migration through the capillary and avoid plugging the microvascular bed. They may also secrete cytokines or vasodilators that transiently dilate capillaries to enable passage. The fact that intracoronary delivery of smaller sized gelatin microspheres (10 μm) can cause microinfarctions supports this cell-specific function.\(^1\) Although mesenchymal stem cells (MSC) that are relatively large in size (≥20 μm) have been detected in extracardiac organs after intracoronary delivery\(^1\); it is not clear whether this occurs with the much larger cardiospheres, enabling escape from capillary entrapment and take up residence in other organs.

The third possibility is that cardiospheres do become entrapped in the microvasculature, but transmigrate across the capillary endothelium before cell entrapment induces microinfarction. It has been shown that after intracoronary injection of MSC, most of the cells transmigrate across the capillary endothelium within the first 30 minutes, but not immediately thereafter.\(^1\) This suggests that the MSC also become entrapped in the microvessels, but as a result of transmigration, do not create microinfarctions. Although MSC differ from cardiospheres in many ways, this mechanism may also take place for cardiospheres.

Finally, in the fourth scenario, the capillary bed is occluded gradually as the cardiospheres are injected, but tissue ischemia and microinfarction do not occur because collateral capillaries maintain tissue perfusion. However, there is probably a threshold where the collateral perfusion becomes insufficient because of excessive cell plugging and microinfarction does occur. Dose-dependent increases in serum cardiac enzyme elevation after intracoronary delivery of single CDC\(^1\) supports this mechanism.

It is likely that all 4 mechanisms take place considering both the efficacy demonstrated in this study, the extracardiac distribution of cells as well as the microvascular plugging reported in other studies using relatively large cells. One recent study that was performed in rats used intracoronary MSC injection for brain cell therapy and reported that cell size, injection speed, and the cell dose are the important factors that determine the occurrence of brain infarction.\(^1\) In this study, 2×10\(^6\) MSC with a diameter of 25 μm resulted in microinfarctions, whereas smaller cells (glial-restricted precursors) with same total cell volume did not cause infarctions. The importance of cell injection speed has also been shown in the pig model of recent myocardial infarction, and a slow rate of injection seems to be better in preventing microinfarctions.\(^1\)

These considerations suggest that there are probably more biological factors to be considered when injecting large diameter cells when compared with smaller cells. It is likely that there is a threshold for the number of cells that can be injected without causing microinfarction, and this also changes by cell type, cell size, and injection speed. The maximum dose tested in Gallet et al\(^1\) did not cause an elevation in cardiac enzymes, thus the threshold dose for intracoronary cardiospheres remains uncertain. Because injecting cell numbers close to the threshold in patients can result in serious safety issues, especially in patients with low cardiac function, elucidating the estimate threshold and examining the efficacy using a submaximal dose with a wide enough safety margin is desirable. In addition, the distribution of injected cells is also of interest because large cells may be associated with greater retention in the heart.

In addition to testing the safety of intracoronary delivery of cardiospheres, Gallet et al\(^1\) compared the therapeutic efficacy of cardiospheres with CDC.\(^1\) This comparison was made using data from previous studies and suggested that cardiospheres were potentially superior to CDC. Although the favorable effect of cardiospheres is certainly promising, indirect comparison contains biases derived from different study design, time points, and cell doses. Thus, future head-to-head studies are needed to determine the superiority of cardiospheres against CDC, and also with other types of stem cells, including purified c-kit+ cells\(^1\) and bone marrow–derived MSC.\(^2\)

The findings from this preclinical study suggest that intracoronary cardiospheres delivery adds another cell therapy option for treating patients with heart failure, but we do not yet know whether this large caliber group of cells offers the best possible therapeutic intervention or which state of myocardial dysfunction will benefit the most from intracoronary cardiospheres therapy. At present, the state of cardiac stem cell therapy may be analogous to the process of making wine (treating diseases) using different types of grapes (stem cells). Some of the grapes are derived from the same type of seed, but differences in growing conditions endow them with different characteristics. We realize that the wines made from these grapes are good; however, we currently do not know which grapes will make the best wines. It might be that a combination of the grapes projects greatest flavors. On the contrary, these wines may have different best matching dishes (ie, type of heart failure). Also, changing the wine manufacturing process (cell delivery) would certainly change the taste. It is likely that a lot of tasting is necessary to determine how to make the best wines.

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Disclosures
None.

References
3 Ishikawa  Intracoronary Injection of Large Cells


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