Does a Newly Characterized Cell From the Bone Marrow Repair the Heart After Acute Myocardial Infarction?

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A new report in *Circulation Research* entitled "S1P–S1PR2 Axis Mediates Homing of Muse Cells Into Damaged Heart for Long-Lasting Tissue Repair and Functional Recovery After Acute Myocardial Infarction" suggests that injection of isolated multilineage-differentiating stress enduring (MUSE) cells into the venous circulation 24 hours after acute myocardial infarction (AMI) reduces scar size and adverse ventricular remodeling and improves cardiac pump function. The authors suggest that bone marrow–derived MUSE cells might be suitable for the treatment of patients who have suffered an AMI.[1]

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AMI leads to the death of the tissue within the infarct core and a surrounding peri-infarct region where myocytes are at risk of subsequent death. Subsequent wound healing is associated with death of myocytes in the peri-infarct zone, causing infarct expansion, the formation of scar tissue, and revascularization of this new tissue. With time the left ventricle dilates and there is progressive depression of cardiac pump function that can lead to heart failure. There is a need for novel therapies that can improve post-AMI remodeling by reducing scar formation, protecting damaged myocytes, and preventing the development of ventricular dilation and depressed pump function. Therapies that regenerate the tissue killed by the ischemic insult are still the holy grail of this field.

Cell therapies for cardiac repair with cells from the bone marrow have been tested in AMI, and although some initially showed great promise in preclinical animal studies,[2] beneficial effects in AMI patients have been small[3] or absent.[4] In the new study in *Circulation Research*,[1] the authors suggest that a subpopulation of bone marrow cells, MUSE cells, repair the rabbit heart after AMI.

In this editorial, I will give my view of the aspects of this work that either have or have not convinced me that MUSE cells induce significant repair after AMI. I will say at the onset that I am far from convinced. I will discuss why and what additional evidence I would need to see before I would recommend that an AMI patient enroll in a clinical trial with these cells.

The most interesting aspects of this new study are the suggestions that when MUSE cells, isolated from rabbit or human bone marrow, are injected into the venous circulation 1 day after AMI (caused by 30 minutes of ischemia followed by reperfusion), they home to the damaged regions of the heart, engraft in the AMI peri-infarct zone where they reduce myocyte death and scar formation and improve cardiac pump function. In addition, the authors argue that MUSE cells rapidly transdifferentiate into new cardiac myocytes. These initial findings, in my view, make these cells worthy of additional study because other putative reparative cells have only had beneficial effects when they were injected directly into the affected region of the coronary circulation[5] or directly into the AMI border zone tissue,[6] requiring more invasive techniques.

In this new study,[1] the authors also determine that the SIP (sphingosine monophosphate)-SIPR2 (sphingosine monophosphate receptor 2) pathway is responsible for MUSE cells to home to and migrate into the damaged heart where they appear to secrete reparative paracrine factors, transdifferentiate into cardiac myocytes that integrate with the myocardium, beat in synchrony with the host heart muscle cells, and form new blood vessels. Interestingly, even when MUSE cells are derived from humans and injected into rabbits, they seem to be immune-privileged so they can escape rejection, survive, proliferate, engraft, and promote beneficial post-AMI healing. If confirmed, these results could lead to a new cell therapy for AMI.

So why am I not excited. First, we have been down this road before, especially with cells derived from the bone marrow.[2,3] Many of these studies have not been validated by others, and subsequent clinical trials have not shown a clinical benefit.[4] Also, because MUSE cells are found in the normal circulation, I wonder why there is little evidence that bone marrow–derived cells enter the damaged heart and transdifferentiate into new cardiac myocytes.[7]

Preclinical AMI therapy studies are best performed in well-validated animal models that recapitulate critical features of the human condition. In the new study,[1] AMI was induced in male rabbits with 30 minutes of ischemia followed by reperfusion. Thirty minutes of ischemia followed by reperfusion likely causes a small necrotic core and large surrounding regions of damaged myocardium that can either die or recover after reperfusion, and the damage is likely to be highly variable.[8] Most studies in larger mammals use longer ischemic times[5,6] with clear necrotic cores. Inadequate characterization of this animal model makes comparisons to other studies and to the human situation challenging. For example, injury was documented with a single 24-hour post-AMI troponin measurement, but it is not clear if this correlates well with the AMI injury.

It is also not clear how animals were randomized to the different treatment groups and how investigators were blinded to treatment. This is important because unintentional bias is

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difficult to eliminate in studies where subjective measurement tools, such as identification of cells positive for different markers (GFP [green fluorescent protein]), are used. This is true in almost all studies of this type, including studies from my laboratory.[6] To protect patients from clinical trials with cells having little therapeutic potential, I personally believe validation studies should always be performed in independent laboratories before translation is considered. How to support and conduct these types of studies is always an issue.[9]

A major finding in this new report is that injection of MUSE cells reduces infarct size by ≈50%, 2 weeks after AMI. The authors do not strongly conclude how they think this might actually happen. They present data (their Figure 6D) supporting the idea that apoptosis of myocytes within the peri-infarct zone is reduced and that some of the MUSE cells transdifferentiate into new cardiac muscle cells. To me the rapidity of the effect strongly supports the idea that MUSE cells protect damaged myocytes in the peri-infarct zone into which they home. To me it is highly unlikely that new myocyte formation has anything to do with a major reduction in infarct area 2 weeks after AMI or to improved function at 2 weeks or 2 months after AMI. The authors estimate there are ≈19 678 MUSE-derived new myocytes 2 weeks after AMI and about that same number at 2 months. They estimate this would represent ≈0.15% of the myocytes in the heart. A similar estimate can be determined from the reported ventricular mass and the average myocyte size using techniques described in previous reports.[10] It is highly unlikely that this small number of putative new myocytes is responsible for the large improvement in cardiac function observed. The authors had an opportunity to address the hypothesis that engrafted and transdifferentiated MUSE-derived myocytes (and blood vessels) directly contribute to the improvements they observed by engineering a death gene into the MUSE cells. However, this strategy was only used to kill the cells immediately after injection and does not give insight into the idea that new tissue directly derived from MUSE cells contributes to the improved function.

The authors present what they think are Ca^{2+} transients (Figure 4) from MUSE-derived new cardiac myocytes infected with GCaMP3 (a calcium indicator that includes GFP [green fluorescent protein], calmodulin, and M13, a peptide sequence from myosin light chain kinase). These cells were directly injected into the myocardial infarction border zone and then studied 2 weeks later. It is unclear why direct injection was required since the MUSE cells home to this region? Using hearts with the preferred method of MUSE cell delivery would have been more convincing? The authors contend that within the 2-week window after direct injection of the MUSE cells, they transdifferentiate into new myocytes, mature, and electrically couple to the parent myocardium. A major concern I have with these data is that there is no way for the reader to determine the temporal relationship between putative Ca^{2+} transients and mechanical activity. An ECG was measured with the fluorescence changes, but the relationships between the QRS complex and fluorescence changes cannot be determined. More importantly, the slowly rising fluorescence signals (Figure 4) are not reminiscent of actual Ca^{2+} transients, which rise to a peak in ≈100 ms and are not temporally identical to contraction.[11] The traces in the report look to me like motion artifacts, which is the major concern with these types of measurements. The control with a few fibroblasts is insufficient in this readers view. Temporal comparisons of ventricular motion and fluorescence signals would have addressed this concern. To me these data are not convincing.

The authors show significant improvements in echocardiographic-derived cardiac structure and function. All of these data are difficult to critically evaluate because no raw data are presented either in the main article or in the supplemental results. Assuming that the data are accurate and reliable, the ejection fraction 2 weeks after AMI in vehicle-treated AMI animals was found to be close to 50% and was nearly 60% in MUSE-treated groups. There are no data presented in animals without AMI, and so these data are difficult to interpret. There are also no raw tracing of ventricular pressure measurements or documentation of the animals age or body weight. The absence of these simple but critical details will make it difficult for comparisons to future studies.

In summary, the most exciting aspect of this new study is that MUSE cells seem to home the infarct border zone when injected into the circulation after AMI. Their major effect seems to be a significant reduction in myocyte death in the myocardial infarction border zone during the first 2 weeks after AMI. There are little convincing data that differentiation of MUSE cells into new myocytes actually occurs and if it does that it plays any role in cardiac repair after AMI. If the actual effect (reduced myocyte death resulting in reduced scar formation) can be validated by others, then future studies should focus on the mechanism for this effect. I think MUSE cells are deserving of additional preclinical study but are not close to being ready for even early-stage clinical trials.

Disclosures

Dr Houser is a named inventor on intellectual property filings on another cell type being tested for cardiac repair and is a cofounder, scientific advisor, and holds equity in Myocard Therapeutics, LLC, a biotech start-up.

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