Microvascular Obstruction by Intracoronary Delivery of Mesenchymal Stem Cells and Quantification of Resulting Myocardial Infarction by Cardiac Magnetic Resonance

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Intracoronary injection of mesenchymal stem cells (MSCs) has been proposed as a potential therapeutic option in repair of the ischemic- or infarct-damaged heart. However, because MSCs are large (rounded up cells are \(\approx 22 \text{ to } 25 \mu\text{m}\) diameter), an important consideration of this approach is the potential of these cells to induce myocardial damage by microvascular obstruction as has been reported previously.¹

We present cardiac magnetic resonance (CMR) images, with corresponding biochemical and histological data from 2 healthy sheep in which 25 or 75\(\times 10^6\) MSCs were injected through an over-the-wire balloon catheter into the mid left anterior descending coronary artery with the balloon inflated briefly before and during the infusion of the cell suspension. The MSC had been prepared as described previously and filtered with a 20-\(\mu\text{m}\) filter.² Serial troponin levels were measured for 24 hours and, weight-adjusted gadolinium (Multithane; 0.1 mmol/kg) was injected 45 minutes before euthanasia. Explanted hearts were imaged by using CMR (1.5T GE scanner) before sectioning and histological analysis. Experimental studies have demonstrated an excellent spatial correlation between the extent of hyperenhancement on CMR delayed-enhancement imaging and areas of myocardial necrosis or scarring at histopathology.³ Performing CMR on ex vivo specimens eliminates the need for cardiac and respiratory gating as well as general anesthesia and animal ventilation, which is complex in a magnetic field. Experimental protocols were approved by our Institute’s animal ethics committee.

Sheep A (25\(\times 10^6\) cells) had a small rise in Troponin T, peaking at 0.21 \(\mu\text{g/L}\). CMR demonstrated no significant late gadolinium enhancement on T1-weighted inversion recovery sequence (TI=300 ms). Histology demonstrated subtle increase in interstitial cellularity predominantly around small vessels. The cells had the appearance of lymphocytes. There was no histological evidence of myocardial infarction. In contrast, Sheep B, which received 75\(\times 10^6\) cells, had a more substantial rise in Troponin T to 0.72 \(\mu\text{g/L}\) and significant late gadolinium enhancement quantified as 13.7 g of 97.1 g (14%) of left ventricular myocardium. As shown in Figure A, there was also a region of no gadolinium uptake within the enhanced segment consistent with persistent microvascular obstruction or “no reflow”. This focal region was moderately hyperintense on T2-weighted imaging and was also evident to the naked eye when the specimen was sectioned (Figure B). This animal histological analysis showed acute myocardial infarction characterized by contraction band necrosis and neutrophilic infiltration (Figure C and D) as well as increased interstitial cellularity similar to that seen in Sheep A. Furthermore, a number of sections showed cells, consistent in size and morphology with MSCs, occluding small vessels.

These cases highlight the potential for intracoronary MSCs to cause microvascular obstruction and resulting myocardial infarction in healthy sheep, an effect that may be less easily distinguished when delivered to injured myocardium. Cell number likely explains the difference in myocardial damage between the 2 sheep presented in this study. We suggest that the effect of other important variables such as the intracoronary infusion protocol or cell preparation can be assessed quantitatively by CMR, ensuring that the delivery of MSC is optimized before use in disease model. Finally, ex vivo CMR of the heart may be a more convenient and accurate method to quantify myocardial infarction in experimental studies compared with conventional tissue-staining techniques. It has been validated against traditional techniques in coregistered slices but has the advantage that the heart remains intact, and infarct volume can be simply calculated from acquired images using a readily available computer algorithm. In contrast, traditional staining techniques require physical slicing of the myocardium, measurement of the area of infarction in a series of slices, and then multiplication of the area by the

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slice thickness to determine a volume for that slice. The volume of infarct for each slice is then summed to calculate the total infarct volume.

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**Disclosures**
None.

**Figure.** CMR and histological data from Sheep B, which received 75 million MSCs through intracoronary injection. A, Gadolinium enhancement seen on CMR of explanted heart with red arrow demonstrating region of persistent microvascular obstruction (or “no reflow”) in the septum. B, Gross appearance of corresponding macroscopic section with focal region of apparent “no reflow” (white arrow). C, Hematoxylin-eosin-stained microscopic sections taken from the region of infarction demonstrate band necrosis (black arrows) and neutrophil infiltration. D, Hematoxylin-eosin staining showing infiltrate by cells resembling lymphocytes. Arrow points to small vessels occluded by cells.

**References**
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