Towards the Second Generation of Skeletal Myoblasts?

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It is now widely accepted that the putative benefits of cell transplantation are hampered by the high rate of cell death that occurs shortly after the procedure. While this event may not be so relevant to bone marrow-derived cellular grafts whose efficacy seems to depend on cell functionality rather than on the absolute numbers of delivered cells, such is not the case for skeletal myoblasts for which a dose-effect relationship has been demonstrated. It thus appears to be sound to speculate that outcomes of myoblast transplantation could be improved if cell survival was enhanced.

Although postdelivery cell death is a complex phenomenon which involves several intricate factors, three of them have been clearly identified: inflammation due to needle-induced tissue disruption, apoptosis due to detachment of anchorage-dependent cells from their extracellular matrix, and ischemia due to both the delivery of cells deprived from their own vascular supply and the poor vascularisation of the target areas of injection. Several studies have already provided evidence for the role of these three factors by showing that tackling any one of them usually improves cell engraftment and left ventricular function.

In the present issue of *Cardiovascular Research*, Aharinejad et al. present data that further support the importance of specifically addressing the ischemic component of postinjection myoblast cell death. Using the classical rat model of myocardial infarction, they basically show that transfecting skeletal myoblasts with the gene encoding colony-stimulating factor (CSF)-1 results in an increase in angiogenesis that is associated with a greater degree of cell engraftment, a better functional outcome and a lesser degree of left ventricular remodelling. The mechanistic link between CSF-1 and these beneficial events is suggested by the expression of CSF-1 mRNAs in myocardial tissue for a few weeks after transplantation while delivery of recombinant human or mouse CSF-1 plasmid is shown to be inefficient, a finding likely explained by its fast clearance from the myocardium. Overall, these results support previous studies that had already provided the proof of principle by showing a similar kind of benefit following administration of slow-release angiogenic factors along with myoblasts or of myoblasts transfected with a single gene, like vascular endothelial growth factor, or with a master gene like hypoxia-inducible factor -1 that controls the expression of multiple downstream angiogenic proteins. Likewise, transfection of myoblasts with stromal-derived factor-1 has been successful in enhancing myocardial homing of angiogenic circulating cells, thereby resulting in greater blood vessel density, improved cellular graft survival and better left ventricular function. However, the present study has distinct methodological features that strengthen its conclusions, such as the large number of included...
animals (170), the extended period of follow-up (3 months), a randomised type of design and a combinatorial approach for blindly assessing outcomes. The only concern that one could raise relates to the reliance on a dye-emitted fluorescence for quantifying cell engraftment whereas the more direct identification of a phenotypic marker specific for myoblasts, like fast skeletal myosin, might have yielded more robust data. However, this comment does not question the overall high quality of this study, which has the additional interest of highlighting the possible specific benefits of CSF-1 through its ability to act in both an autocrine manner by stimulating myoblast proliferation and in a paracrine manner by attracting macrophages. This, in turn, seems to affect the expression pattern of metalloproteases in a way that could beneficially impact on postinfarction left ventricular remodelling.

Once data have been deemed convincing, which is clearly the case for those of this study, the next issue is to figure out how they can be exploited therapeutically. Indeed, myoblast transplantation may now be approaching a crossroad. Clearly, the expectations raised by the consistently positive results of experimental studies have not been met by those of the first randomized controlled clinical trials, as none of them, regardless of whether myoblasts were delivered by a catheter (P.W. Serruys, Communication at the 2008 American College of Cardiology) or surgically⁸, have successfully met their primary end point, usually an improvement in global and/or regional left ventricular function. These studies, however, have yielded some hints of efficacy that probably reflect the paracrine effect of cell-derived cytokines that can act upon various endogenous pathways and result in increased angiogenesis (as shown by the comparison, in the present study, between the myoblast-alone and the control groups), reduced fibrosis and, possibly, rescue of host cardiomyocytes. Clearly, however, these effects are not of sufficient magnitude to translate into clinically meaningful improvements in heart failure patient outcomes. From these considerations, two strategic options can be considered.

The “aggressive” option consists of attempting a true regeneration of scarred myocardium by populating it with cells featuring the capacity to functionally integrate into the recipient heart. The increased recognition that adult stem cells have a much more limited plasticity than initially thought has progressively led to the assumption that cells that would best fulfill this requirement were those which recapitulate a cardiomyogenic developmental program. The choice is then fairly limited and the uncertainties regarding the persistence of cardiac stem cells in adulthood⁹ lead to the conclusion that appropriately cardiac-specified human embryonic stem cells (ESC) are likely the best candidates for making new myocardial tissue. Despite the ethical and technical issues associated with this cell type, there is now
fairly robust evidence that ESC-derived cardiac progenitors engraft into infarcted myocardium, can connect with the host cardiac cells and thus contribute to ameliorate pump function without causing teratoma\textsuperscript{10} provided that they have been reliably sorted prior to implantation so as to discard still undifferentiated cells. However, the clinical use of these progenitors, whether derived from true ESC or induced pluripotent cells\textsuperscript{11}, may appear to be still far-reaching, and thus an alternate, more conservative approach may be justified.

This “conservative” approach consists of building an improved cell therapy product on the basis of the existing bulk of data collected over the last decade. The study by Aharinejad et al. fits this paradigm. Whether CSF-1 is the best candidate remains to be validated in a more clinically relevant, large animal model, but is it yet noteworthy that the finding that a short-lived gene expression is still sufficient to trigger beneficial effects (by 3 months, in this study, CSF-1 mRNA levels were no longer changed) should be helpful for overcoming several of the vector-associated safety concerns. Thus, while they do not preclude additional improvements like incorporation of cells into bioresorbable scaffolds providing them a more physiological microenvironment, the results presented in this paper might well outline the frame of a second generation of “optimised” myoblasts carrying the hope of improved clinical outcomes in transplanted patients.
References


