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Fortune Favors the Prepared: Safety and Efficacy of Allogeneic Hypoxia Preconditioned Mesenchymal Stromal Cells in Primates

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The intrinsic regenerative capacities of the adult mammalian heart are believed to be limited and insufficient to compensate for major cell loss upon injury. Transplantation of cells bearing regenerative potential emerged as a possible therapeutic strategy more than a decade ago; however, investigators continue searching for the ideal cell and implantation strategy.¹ Mesenchymal stromal cells (MSC) are among the most widely used cell types for regeneration studies.² MSC are multipotent, but rigorous *in vivo* proof of their stem cell identity is still lacking. Residing in the perivascular space of nearly all tissues, MSC are most commonly retrieved from bone marrow or adipose tissue aspirates. MSC are immune evasive and exhibit immunomodulatory properties,³ importantly making them suitable for possible allogeneic use. MSC are identified by a defined panel of surface markers in the absence of hematopoietic markers and by their *in vitro* tri-lineage differentiation potential into osteogenic, adipogenic and chondrogenic lineages.⁴ Irrespective of these criteria, MSC represent a heterogeneous population and their properties depend on a variety of factors, which include donor and tissue of origin as well as isolation and culturing techniques. In particular the choice of culturing media, supplements and oxygen supply may greatly influence their characteristics, which can be exploited to modify their regenerative potential.

“Fortune favors the prepared mind” (Louis Pasteur, 1854), as biology favors the prepared cell. Besides molecular or pharmacologic manipulation and genetic engineering, culturing of MSC under hypoxic conditions prior to transplantation is one of the strategies to enhance their viability and engraftment and to modify their secretome, thus promoting anti-apoptotic, anti-inflammatory and pro-regenerative properties.⁵ Hypoxic preconditioning has the advantage that it is broadly available, easily applicable and free of safety concerns inherent

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to pharmacological or genetic manipulation. Nevertheless, because hypoxic preconditioning profoundly alters cell properties and their interaction with the microenvironment, it may also affect their safety profile.

In the present issue, Hu et al. undertook the great effort to investigate whether transplantation of allogeneic hypoxia preconditioned MSC is safe and efficacious compared to MSC cultured under ambient conditions in a randomized trial in monkeys subject to acute myocardial infarction.⁶ MSC were isolated from the bone marrow, labeled with green-fluorescent protein by lentiviral transfection and cultured either under ambient conditions (21% O₂, N-MSC) or under hypoxic conditions (0.5% O₂, HP-MSC) for 24 hours. Cells (1×10⁷ per heart) were injected intramyocardially at five different sites into the infarct border zone 30 minutes after ligation of the left anterior descending coronary artery. Applying a thought-through study design employing magnetic resonance imaging (MRI) and positron emission tomography (PET) to determine cardiac function and metabolism as well as telemetry and electrophysiology to monitor rhythm and assess proarrhythmogenic potential, the investigators report improved viability, function and remodeling of HP-MSC injected as compared to N-MSC- or vehicle-injected monkey hearts. These beneficial effects occurred in the absence of arrhythmogenic complications and were associated with enhanced cell engraftment, cardiomyocyte proliferation, increased vascularization and less apoptosis of intrinsic cardiac cells. Hu et al. did not, however, observe neo-cardiomyogenesis, and persistence of engrafted MSC was short-lived.

Given that the benefits of hypoxia preconditioning of MSC have previously been explored in various disease models with positive outcome,⁷ how does this study expand current knowledge? Large animal studies have proven informative regarding safety and outcome of cardiac cell therapy in clinical trials,⁸ but primate studies are rare. Not only are they expensive and time-consuming, but also they require special expertise and underlie tight regulation. With respect to hypoxia preconditioning in MSC, only a single study exists in chronic ischemic heart failure in pigs (cell transplantation 30 days after infarction). This prior worked reported improvements in left-ventricular function in animals receiving allogeneic HP-MSC as compared to N-MSC.⁹ The study by Hu et al. is the first study in primates and the first large animal study in acute myocardial infarction. Cynomolgus monkeys (*macaca fascicularis*), which belong to the Macaques family, are frequently used for toxicology and preclinical safety studies and their anatomy and physiology share great similarities with humans. They are also well suited for cardiac imaging¹⁰ and telemetry testing,¹¹ which was employed by the investigators, who chose a clinically relevant study design to monitor arrhythmogenic complications and cardiac metabolism and function *in vivo* in a similar fashion to a clinical trial in humans. The improvements in myocardial viability and function in the absence of significant arrhythmias indeed raise hope that HP-MSC therapy may be safe and efficient for potential use in humans. However, an additional strength of large animal studies is the possibility of long-term follow-up. This is particularly relevant in view of clinical studies in humans, in whom long-term outcome is the utmost concern and importance. Hu et al. opted for long-term follow-up and data are reported for up to 270 days. Although the number of animals is too small to draw definitive conclusions, the effect of HP-MSC therapy appeared to diminish over time from an increase of roughly 10% in left ventricular ejection fraction (LVEF) day 90 versus day 3, to 2.8% day 270 versus day

3 post-infarct and treatment. The authors have stated that follow-up is still ongoing and long-term outcomes will be of great interest in view of future clinical trials using hypoxia preconditioned MSC in patients.

Large animal models while important in the development of novel therapeutics, are not traditionally the first model systems used for deriving mechanistic insights and to develop new biological concepts. Nevertheless, Hu et al. provide sufficient data to mechanistically support the beneficial effects of HP-MSC on cardiac remodeling and function seen in the primate heart. Although their findings are consistent with prior findings in MSC, there are several points worth considering in greater detail: MSC have been shown to exhibit cardiomyogenic and vasculogenic differentiation potential *in vitro* and *in vivo*, and along with gain in cardiac function, true regeneration of lost cardiac muscle remains among the primary goals of cell transplantation studies, particularly for sustained long term cardiac improvement. However, *bona fide* differentiation of MSC is low and their beneficial effects are mostly attributed to their paracrine activity.¹² The lack of neomuscularization observed in the study by Hu et al., therefore, is not of great surprise. In light of recently reported neomuscularization of large areas of injured macaque hearts based on transplantation of human embryonic stem cell-derived cardiomyocytes,¹³ though, the role of MSC must be critically evaluated. Efficient differentiation of MSC to meaningfully contribute to neomuscularization of the infarcted heart – if possible at all – may come with important downsides. MSC may not be immunoprivileged after all, but rather immune evasive,³ and differentiation markedly alters their immunogenic properties. The increased expression of the immunogenic major histocompatibility complex (MHC) Ia and II and the reduced expression of the immunosuppressive MHC Ib observed in MSC undergoing differentiation into any of the three major cardiac lineages (endothelial, myogenic or smooth muscle cell),¹⁴ will ultimately jeopardize this immune evasiveness, making immunosuppression mandatory. In addition, the large bulk of cells needed to engraft bears a much higher risk of electrical destabilization than the <1% of 1×10^7 injected cells engrafted in the study by Hu et al, irrespective of cell type. Indeed, all animals experienced arrhythmias for up to four weeks after transplantation in the study by Chong et al,¹³ but no significant arrhythmias were observed in association with HP-MSC therapy.⁶

Rather than neomuscularization, the observed beneficial effects reported by Hu et al. likely arise from the paracrine properties of MSC, which were augmented by hypoxia preconditioning. HP-MSC expressed higher levels of hypoxia-inducible factor 1 α (HIF1 α), erythropoietin (EPO) and angiopoietin-1 (Ang-1), exhibited enhanced apoptosis resistance *in vitro*, and more efficiently induced tube formation of endothelial cells than N-MSC. EPO and Ang-1 are known constituents of the MSC secreted proteome, and have previously been implicated in angiogenesis, fibrosis inhibition and apoptosis protection.⁵ HIF1 α is the oxygen-sensitive subunit of the transcription factor HIF1, a master regulator of the hypoxia response. Activity of HIF1 is induced under hypoxic conditions through changes in HIF1 α mRNA and protein expression, subsequently enhancing transcription of a variety of growth factors and cytokines, many of which can be found in the MSC secretome, including EPO and Ang-1.¹⁵

Similarly to previous studies using MSC, the beneficial effects of HP-MSC observed by Hu et al. outlasted the traceability of engrafted cells in the heart, a phenomenon also referred to as “hit and run”- mechanism. How precisely MSC effects are maintained long after the cells disappear is poorly understood. In addition to proteins, the MSC secretome contains nucleic acids including mRNAs and miRNAs, and lipids, packaged in exosomes or microvesicles. Recent work demonstrates that such microvesicles are capable of transferring mRNA or proteins into recipient host cells thereby altering protein expression¹⁶ or epigenetic programming,¹⁷ particularly the latter offering a possible explanation for some of the long-lasting effects of MSC. Let alone that the MSC secretome is far from being identified, much more research is needed to define whether and under what circumstances comparable mechanisms may occur in the myocardium. In addition, hypoxia preconditioning could theoretically alter each of the secretome’s components. Collectively, the cardiovascular field will be required to dig deeper into the molecular mechanisms underlying the cardiac benefits of transplanted cells if cell therapy is to become a real treatment option in the future.

In the interim, the findings of Hu et al. remain an important step forward (Figure). MSC may indeed be ideal carriers of a thus far poorly understood secretome, the efficacy of which can further be enhanced by hypoxia preconditioning. In the not too distant future, we may have the possibility of cell-free delivery of a beneficial cocktail to induce cardiac repair, and identifying the critical components of this cocktail as well as the biological mechanisms underlying their effects will emerge from continued studies such as Hu et al. Biomedicine has long taught that there are no shortcuts when it comes to emerging therapies, and continued studies with long-term outcomes data and mechanistic studies are awaited with great eagerness.

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References

1. Behfar A, Crespo-Diaz R, Terzic A, Gersh BJ. Cell therapy for cardiac repair—lessons from clinical trials. *Nat Rev Cardiol.* 2014; 11:232–246. [PubMed: 24594893]
2. Karantalis V, Hare JM. Use of mesenchymal stem cells for therapy of cardiac disease. *Circ Res.* 2015; 116:1413–1430. [PubMed: 25858066]
3. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: Immune evasive, not immune privileged. *Nat Biotechnol.* 2014; 32:252–260. [PubMed: 24561556]
4. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy.* 2006; 8:315–317. [PubMed: 16923606]
5. Konala VB, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: A new paradigm for cell-free regeneration. *Cytotherapy.* 2016; 18:13–24. [PubMed: 26631828]
6. Hu X, Xu Y, Zhong Z, et al. A large-scale investigation of hypoxia-preconditioned allogeneic mesenchymal stem cells for myocardial repair in non-human primates: Paracrine activity without remuscularization. *Circ Res.* 2016

7. Das R, Jahr H, van Osch GJ, Farrell E. The role of hypoxia in bone marrow-derived mesenchymal stem cells: Considerations for regenerative medicine approaches. *Tissue Eng Part B Rev*. 2010; 16:159–168. [PubMed: 19698058]
8. van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P, van Belle E, Gyongyosi M, Sluijter JP, Cramer MJ, Doevendans PA, Chamuleau SA. Human relevance of pre-clinical studies in stem cell therapy: Systematic review and meta-analysis of large animal models of ischaemic heart disease. *Cardiovasc Res*. 2011; 91:649–658. [PubMed: 21498423]
9. Jaussaud J, Biais M, Calderon J, Chevaleyre J, Duchez P, Ivanovic Z, Couffinhal T, Barandon L. Hypoxia-preconditioned mesenchymal stromal cells improve cardiac function in a swine model of chronic myocardial ischaemia. *Eur J Cardiothorac Surg*. 2013; 43:1050–1057. [PubMed: 23100292]
10. Sleeper MM, Gaughan JM, Gleason CR, Burkett DE. Echocardiographic reference ranges for sedated healthy cynomolgus monkeys (*macaca fascicularis*). *J Am Assoc Lab Anim Sci*. 2008; 47:22–25. [PubMed: 18210994]
11. Gauvin DV, Tilley LP, Smith FW Jr, Baird TJ. Electrocardiogram, hemodynamics, and core body temperatures of the normal freely moving cynomolgus monkey by remote radiotelemetry. *J Pharmacol Toxicol Methods*. 2006; 53:140–151. [PubMed: 16143547]
12. Gneccchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*. 2008; 103:1204–1219. [PubMed: 19028920]
13. Chong JJ, Yang X, Don CW, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature*. 2014; 510:273–277. [PubMed: 24776797]
14. Huang XP, Sun Z, Miyagi Y, McDonald Kinkaid H, Zhang L, Weisel RD, Li RK. Differentiation of allogeneic mesenchymal stem cells induces immunogenicity and limits their long-term benefits for myocardial repair. *Circulation*. 2010; 122:2419–2429. [PubMed: 21098445]
15. Semenza GL. Hypoxia-inducible factor 1 and cardiovascular disease. *Annu Rev Physiol*. 2014; 76:39–56. [PubMed: 23988176]
16. Tomasoni S, Longaretti L, Rota C, Morigi M, Conti S, Gotti E, Capelli C, Introna M, Remuzzi G, Benigni A. Transfer of growth factor receptor mrna via exosomes unravels the regenerative effect of mesenchymal stem cells. *Stem Cells Dev*. 2013; 22:772–780. [PubMed: 23082760]
17. Liu S, Liu D, Chen C, Hamamura K, Moshaverinia A, Yang R, Liu Y, Jin Y, Shi S. Msc transplantation improves osteopenia via epigenetic regulation of notch signaling in lupus. *Cell Metab*. 2015; 22:606–618. [PubMed: 26365178]

Figure. Proposed model of hypoxia preconditioned (HP) MSC action in the injured primate heart according to the study by Hu et al

For details see text. Remaining open questions include whether the beneficial effects of HP-MSC delivery translate into meaningful long-term improvements of cardiac remodeling and function beyond the observation duration of 90 days. From a cell biological perspective, more work is needed with respect to the composition and identification of active constituents of the MSC secretome and how precisely these are modulated by preconditioning. Also the molecular mechanisms of the target/host cell response require further investigation.