JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 12, 2023

EDITORIAL COMMENT

Can Mesenchymal Stem Cell–Derived Therapeutics Protect the Developing Brain During Cardiac Surgery?*

Georg Hansmann, MD, PhD

Neonates, infants, and children with congenital heart disease (CHD) have a high incidence of prenatally or postnatally acquired brain abnormalities. Moreover, they are at particular risk for neurologic impairment due to unfavorable hemodynamics and/or stress injury during cardiac surgery—on full-flow cardiopulmonary bypass (CPB) or in deep hypothermic cardiac arrest (DHCA). Oxidative and inflammatory stress in the central nervous system caused by CPB results in prolonged microglia activation and cortical dysmaturation in the developing brain, thereby contributing to neurodevelopmental impairment in children with CHD. Postoperative systemic-inflammatory response syndrome can be triggered by CPB and may worsen the aforementioned alterations.

Based on their regenerative, immune-modulatory (anti-inflammatory/proresolving) and antifibrotic potential, and their key role for organ homeostasis, wound healing, and proper aging, mesenchymal stem cells (MSCs) (also called "mesenchymal stromal cells") and their products (conditioned media [CM]; extracellular vesicles [EVs]) have been proposed as extremely promising therapeutics, especially for debilitating diseases with an unmet medical need.[1] However, although MSCs have been the subject of clinical trials for more than 30 years, the outcomes have fallen short of the high expectations that arose from encouraging preclinical animal studies in a wide array of disease models. To date, despite published efficacy of MSC administration in multiple preclinical studies, it has not been demonstrated in any preclinical or clinical study that exogenously administered MSCs do transdifferentiate and engraft into the heart (<0.002%), lung, or brain, at substantial quantity or long term.

Whereas certain priming measures in MSCs might increase their transdifferentiation, engraftment, and anti-inflammatory/proregenerative potency,[2] the major beneficial effects of MSCs are widely accepted to be of paracrine nature (secretion). The MSC secretome, harvested as MSC-CM (cell culture supernatant), contains several key factors boosting regeneration, autophagy, immunomodulation/anti-inflammation (monocyte/macrophage reprogramming), and mitochondrial function; those beneficial CM components include, for example, the proteins APOE, LRP1, P38, as well as PGE2 (in human umbilical cord [HUC] MSC-CM),[3] and EVs, that is, microvesicles (ID <1,000 nm)[4] and exosomes (ID <150 nm), containing multiple RNAs, proteins, and lipid rafts.[4] These CM components, applied either as an "unfiltered CM cocktail"[3] or highly concentrated EVs, are probably equally effective as, if not superior to, MSC infusions.

MSCs are immunologically inert, and as such, pooled "unmatched" healthy donor, bone marrow (BM)-MSCs are already used in patients to treat therapy-resistant chronic inflammatory bowel or graft-versus host disease. However, (pre)clinical evidence for their efficacy in preventing or reversing brain damage/neurologic impairment is scarce.

In this issue of the *JACC: Basic to Translational Science*, Sarkislali et al[5] explored whether BM-MSC delivery to the early postnatal brain at the time of

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Hannover, Germany; and the European Pediatric Pulmonary Vascular Disease Network, Berlin, Germany.

The author attests they are in compliance with human studies committees and animal welfare regulations of the author's institution and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.08.012

cardiac surgery inhibits neuronal damage through suppression of inflammation, microglia activation, and neuronal apoptosis, in vivo. The investigators applied an established juvenile piglet model and found that arterial delivery of human BM-derived MSCs via CPB minimizes microglial activation and neuronal apoptosis in the central nervous system, with subsequent rescue of behavioral and structural impairments after neonatal CPB. There are 7 major findings in this study: 1) BM-MSCs are delivered efficiently via the CPB arterial cannula to all organs, including the brain; 2) the delivered BM-MSCs disappear from the brain within 4 weeks (ie, no long-term MSC tissue engraftment); 3) microglial number and activation decrease 4 weeks after CPB; 4) neuronal cell death is reduced; 5) neurologic function and brain structure improved post-CPB; 6) Jak-Stat3 (which is known to drive microglial inflammation and neuronal cell death) and inhibitor of antiapoptotic nuclear factor-κB signaling (*Nfkbia*) are down-regulated on the messenger RNA level; and 7) the MSC-EV microRNA-21-5p is identified in silico as a potential key suppressor of STAT3-mediated microglial (over)activation and neuronal apoptosis.

Indeed, Sarkislali et al[5] found microRNA-21-5p sequence alignment with the IL6ST 3′ untranslated region, a gene known to encode a signal transducer of the IL6/JAK/STAT3 signaling cascade. MicroRNA-21-mediated down-regulation of IL6ST is predicted to inhibit STAT3 activation by dampening signal transduction through the IL6 receptor complex. Although the investigators did not find differentially expressed genes in the TGFB pathway in their bulk cortical tissue RNA-sequencing data, they did not study the cortical proteome or secretome. It is possible that, for example, the noncanonical, proproliferative TGFB-STAT3-FOXO1 pathway is activated post-transcriptionally by CPB in the microglia of the developing brain (STAT3 phosphorylation). Importantly, overactivated microglia can promote neurotoxicity.

Consistently, IFN-γ-primed rat BM-MSCs had been shown to induce functional recovery following ischemic stroke via regulation of inflammation and oligodendrogenesis in the rat middle cerebral artery occlusion model,[2] making MSC-derived therapeutics attractive beyond CPB-induced neurologic injury. Several translational points may be considered to increase the neuroprotective potential of human MSC-derived therapeutics even further:

1) Given that the major beneficial effect of MSCs is of paracrine nature, noncellular MSC approaches, such as “unfiltered” MSC-CM may be not only safer but also more efficient than attempted MSC transplantation that comes along with unclear, only temporarily tissue engraftment, and low but not negligible iatrogenic stroke risk when administering MSCs intravascularly.
2) Neonatal tissue-derived MSCs (HUC, placenta) have been reported to have superior regenerative capacity and superior long-term propagation compared to MSCs isolated from adult human tissues (BM, peripheral blood, adipose tissue). A comparative single-cell RNA-sequencing transcriptomic analysis of therapeutic HUC MSCs vs nontherapeutic human umbilical vein endothelial cells, and the accordingly successful first-in-human HUC MSC-CM treatment of a child with severe cardiovascular disease, have recently been published.[3] Hence, there is a good rationale to test delivery of HUC MSCs and/or their secreted products (CM or EVs) in the juvenile piglet CPB and rodent/porcine stroke models.
3) Repetitive dosing of MSC-EVs within a few days has been shown to be superior to large single dosing. Moreover, MSC or MSC-product administration may be even more neuroprotective when started 24-48 hours before CPB-cardiac surgery. Repetitive dosing of HUC MSC-CM has recently been conducted in a clinical case of severe pulmonary arterial hypertension,[3] thereby driving regeneration, mitochondrial function, anti-inflammation/immunomodulation, and autophagy, counteracting inflammation, pulmonary artery smooth muscle cell proliferation, and fibrosis. Hence, for future animal or clinical phase 1/2 studies, intravenous or intra-arterial MSC-derived therapeutics should probably be best administered in a repetitive fashion, starting well before the expected neurologic insult.
4) Route of administration of MSCs during CPB via the arterial cannula may be beneficial, but it is not clear that this approach is superior vs peripherally venous or centrally venous MSC/MSC product application. For repetitive dosing of MSCs/MSC products, the intravenous route appears most easily accessible. Venously injected stem cells, including MSCs, stay longer in the lungs than in other organs, but it has not been shown that these MSCs engraft; it is unclear what the role of these transient MSCs in the lungs are and whether they are actually vital and functional. A lower brain distribution after peripheral or centrally venous intravenous injection of stem cells, compared to the arterial delivery via CPB, has not been demonstrated in humans, so the best route of MSC administration remains to be determined.

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 12, 2023
DECEMBER 2023:1536–1538

Of note, Sarkislali et al[5] performed all bulk RNA-sequencing analyses using cortical tissues after CPB with DHCA, whereas full-flow CPB without DHCA was used in the survival experiments to comply with the 3R—replacement, reduction, and refinement—rules for large animal experimentation. The group had previously shown similar changes between full-flow CPB and CPB with DHCA in prolonged microglia expansion and cortical dysmaturation at post-CPB week 4. The investigators omitted a sham surgery group to minimize and reduce use of large animals. It is noteworthy that the piglets used did not have CHD and therefore likely had normal hemodynamics before undergoing CPB (without intracardiac surgery). Thus, extrapolation of outcome from this juvenile piglet model to very young CHD patients undergoing cardiac surgery on CPB is somewhat limited. In addition, the mechanistic insights of this study mainly result from in silico analysis. Future experimental validation of these in silico targets is needed to provide conclusive evidence for their role in the therapeutic effects of BM-MSCs.

Taken together, newly acquired brain damage is commonly recognized after cardiac surgery with state-of-the art imaging, but mild changes may remain undetected. Further refinement of pediatric cardiac surgery, including adjunct therapies before, during, and after CPB surgery, will likely improve neurodevelopmental outcomes in CHD. Sarkislali et al[5] should be congratulated for conducting a landmark study in the field of CHD, CPB, and translational MSC application. Hopefully, this work will lay the land for future experimental validation and meaningful clinical studies on MSC-derived prevention of both brain injury and neurodevelopmental impairment in young patients undergoing cardiac surgery.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr Hansmann is an inventor on 2 submitted patent applications related to MSC-derived therapeutics (EP22177263.5, PCT/EP2023/064627).

ADDRESS FOR CORRESPONDENCE: Prof Dr Georg Hansmann, Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. E-mail: georg.hansmann@gmail.com.

REFERENCES

1. Pittenger MF, Discher DE, Peault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med*. 2019;4:22.

2. Tobin MK, Stephen TKL, Lopez KL, et al. Activated mesenchymal stem cells induce recovery following stroke via regulation of inflammation and oligodendrogenesis. *J Am Heart Assoc*. 2020;9(7):e013583.

3. Hansmann G, Chouvarine P, Diekmann F, et al. Human umbilical cord mesenchymal stem cell-derived treatment of severe pulmonary arterial hypertension. *Nat Cardiovasc Res*. 2022;1:568–576. https://doi.org/10.1038/s44161-022-00083-z

4. Shah R, Patel T, Freedman JE. Circulating extracellular vesicles in human disease. *N Engl J Med*. 2018;379(10):958–966.

5. Sarkislali K, Kobayash K, Sarić N, et al. Mesenchymal stromal cell delivery via cardiopulmonary bypass provides neuroprotection in a juvenile porcine model. *J Am Coll Cardiol Basic Trans Science*. 2023;8(11):1521–1535.

KEY WORDS cardiac surgery, cardiopulmonary bypass, congenital heart disease, mesenchymal stromal cells, neuroprotection