Is a Part Better than the Whole for Cell-based Therapy for Acute Respiratory Distress Syndrome?

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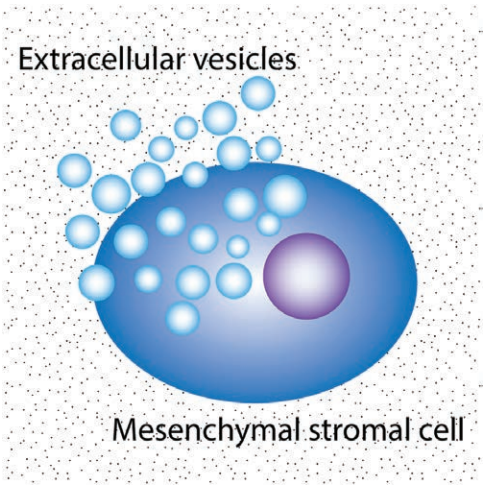
Acute respiratory distress syndrome (ARDS) is a devastating clinical condition common in patients with respiratory failure in the intensive care unit. It is associated with high mortality rates and long-term physical and psychologic dysfunction among survivors.[1] Based on promising preclinical data, clinical trials with mesenchymal stromal cells for ARDS are underway and constitute a new therapeutic approach.[2] Although no safety issues have been identified,[2] there remain some concerns with giving large numbers of live mesenchymal stromal cells intravenously, up to 10 million cells per kilogram per dose, in critically ill patients with systemic inflammation and pulmonary vascular dysfunction. In the current issue of Anesthesiology, Varkouhi *et al.*[3] tested the therapeutic use of extracellular vesicles released by human umbilical cord–derived mesenchymal stromal cells in a well-established rat model of severe *Escherichia coli* bacterial pneumonia as an alternative to giving live cells, bypassing these biologic concerns.



"[Extracellular vesicles,] once considered cellular debris, or … biomarkers of disease progression, … are now recognized as important mediators of cellular communication and function."

Extracellular vesicles are a heterogeneous group of anucleate vesicles with a diameter of 50 to 1,000 nm that are released from intracellular compartments as exosomes or by budding off the plasma membrane as microvesicles in response to diverse physiologic or pathophysiologic stimulus. Extracellular vesicles comprise of exosomes, microvesicles, and apoptotic bodies released by endogenous living and dying cells. They were once considered cellular debris or, more recently, as biomarkers of disease progression, though they are now recognized as important mediators of cellular communication and function.[4] Through its cargo containing bioactive molecules such as proteins, messenger RNAs, microRNAs and organelles (*i.e.*, mitochondria), and its interaction with target cells, extracellular vesicles are recognized as having significant biologic properties.[5]

Multiple preclinical studies have demonstrated the therapeutic potential with mesenchymal stromal cells for acute lung injury in both small and large animal models. The therapeutic effects of mesenchymal stromal cells appeared to arise in part from the secretion of growth factors such as keratinocyte growth factor, antiinflammatory products such as prostaglandin E2 or lipoxin A4, antipermeability factors such as angiopoietin-1, and antimicrobial products such as lipocalin2.[6] Although the safety profile of mesenchymal stromal cells in clinical trials has been excellent to date, some concerns still persist concerning their tumorigenic potential.[6] As an alternative to live cells, multiple investigators have reported beneficial effects of mesenchymal stromal cell–derived conditioned medium or extracellular vesicles for various organ injury models, including a recent study in an *ex vivo* perfused human lung preparation that was injured with live bacteria.[7] In the current study, similar to mesenchymal stromal cells,[8] Varkouhi *et al.*[3] found that the intravenous administration of either mesenchymal stromal cell–derived extracellular vesicles or extracellular vesicles released from mesenchymal stromal cells primed with interferon-γ increased survival in adult male Sprague–Dawley rats injured with *E. coli* pneumonia at 48 h. Pretreatment with interferon-γ was used to upregulate immune-related genes in mesenchymal stromal cells, including major histocompatibility complex, costimulatory

Image: J. P. Rathmell.

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molecules such as CD80 or CD86, and indoleamine 2,3 dioxygenase, to potentially increase the antimicrobial activity of the released extracellular vesicles. The use of interferon-γ was similar to the strategies used by other investigators to pretreat mesenchymal stromal cells, such as with polyinosinic-polycytidylic acid, carbon monoxide, or hypoxia,[9] to enhance the therapeutic properties of the cells and the released extracellular vesicles. Surprisingly, only interferon-γ–primed mesenchymal stromal cell extracellular vesicles but not naïve mesenchymal stromal cell extracellular vesicles reduced alveolar-arterial oxygen difference, lung protein permeability, and alveolar inflammation and enhanced endothelial nitric oxide production in the injured lung compared with controls. The lack of benefit of naïve mesenchymal stromal cell extracellular vesicles may reflect a "survival bias" as suggested by the authors because one-third of the control animals did not survive to 48 h, the time period where all the biologic measurements were made in the surviving rats. However, both interferon-γ–primed and naïve mesenchymal stromal cell extracellular vesicles increased *E. coli* bacteria phagocytosis and killing in human THP-1–derived macrophages (a frequently used substitute cell line for blood macrophages) *in vitro,* which was consistent with the findings from previous investigations.[10]

There are some limitations to these studies which will require further research to more clearly define the potential therapeutic use of mesenchymal stromal cell extracellular vesicles in ARDS. (1) Although interferon-γ–primed mesenchymal stromal cell extracellular vesicles increased macrophage phagocytosis of *E. coli* bacteria *in vitro* and numerically decreased the bacterial colony-forming unit levels in the injured alveolus *in vivo*, mesenchymal stromal cell extracellular vesicles were administered only 30 min after initiation of injury. Experiments are needed with administration at later time points to determine whether the phenotype of interferon-γ–primed mesenchymal stromal cell extracellular vesicles will have therapeutic value once the lung injury has been present for a longer period of time, similar to what would be the case in the clinical setting of ARDS. (2) To determine the mechanisms for the priming effect of interferon-γ, additional studies are needed to assess the messenger RNA, microRNA, and protein content of interferon-γ–primed mesenchymal stromal cell extracellular vesicles compared with naïve extracellular vesicles. (3) Before any clinical trial, any differential effects of mesenchymal stromal cell extracellular vesicles based on sex need to be elucidated. (4) And, perhaps more importantly, priming mesenchymal stromal cells with interferon-γ changed the size distribution of the released extracellular vesicles, emphasizing the need to understand whether exosomes or microvesicles was driving the beneficial response.

Despite these limitations, the current study provides more evidence that extracellular vesicles may be a viable alternative to using live mesenchymal stromal cells for treatment of ARDS. The benefits include ease of storage, avoiding the need for the cell preservative dimethyl sulfoxide and the need for a bone marrow transplant facility, avoidance of using live cells that could be associated with as yet unknown safety issues, the potential to modify extracellular vesicles with pretreatment of the mesenchymal stromal cells to enhance the therapeutic effects, and the potential to administer higher and more frequent doses than may be possible with live mesenchymal stromal cells. However, the major challenge for clinical translation of extracellular vesicle therapy is how to scale up the production of mesenchymal stromal cell extracellular vesicles because the potency is approximately one-tenth of the mesenchymal stromal cells themselves.[10] Given that a typical mesenchymal stromal cell dose is 10 million cells per kilogram or 700 million cells for a 70-kg patient,[3] future clinical trials with mesenchymal stromal cell extracellular vesicles may require isolating extracellular vesicles released from up to 7 billion cells per patient, which may be logistically impossible. Studies are ongoing to determine whether the source of the mesenchymal stromal cells, whether from the umbilical cord, adipose tissue, or bone marrow, or the method of priming, interferon-γ, polyinosinic-polycytidylic acid, carbon monoxide, and hypoxia,[9] may reduce the number of mesenchymal stromal cells that are required to generate enough extracellular vesicles for a therapeutic response. However, more research will be required to determine whether these priming methods will substantially reduce the number of mesenchymal stromal cells needed to generate extracellular vesicles for clinical application, and there will also likely be regulatory issues to be satisfied with any priming method used to modify mesenchymal stromal cells and the extracellular vesicle products.

Despite extensive research and numerous preclinical studies identifying various biologic mediators, there are no specific pharmacologic therapies for ARDS, and treatment is largely limited to supportive care and lung protective ventilation. The article by Varkouhi *et al.*[3] further adds to the biologic and clinical rationale to study mesenchymal stromal cell–derived extracellular vesicles as a promising new therapeutic approach for ARDS.

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Competing Interests

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