Artigo original

Cell Therapy in Acute Lung Injury.

Terapia Celular em Lesão Pulmonar Aguda.

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ABSTRACT

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) continue to be major causes of morbidity and mortality in critically ill patients. Investigations indicate that stem cells represent a viable therapeutic option for patients with ALI/ARDS.

Recent studies have described the mechanistic pathways of stem cells in critical illness. This article reviews the use of stem cells as a potential for exogenous cell-based therapy in ALI.
Further studies are needed in order to fully elucidate the mechanisms involved in the immunomodulatory activities of stem cells, as well as in their mobilisation and tissue engraftment.

Keywords: acute lung injury; sepsis; stem cell transplantation.

RESUMO

A lesão pulmonar aguda (LPA) e a síndrome do desconforto respiratório agudo (SDRA) permanecem como uma das principais causas de morbidade e mortalidade em pacientes internados em unidades de terapia intensiva. Diversos estudos demonstram que as células-tronco podem representar uma opção terapêutica viável para pacientes com LPA/SDRA.

Estudos recentes têm descrito as vias mecanísticas das células-tronco em doenças graves. Este artigo faz uma revisão crítica do uso de células-tronco em LPA como um potencial para a terapia celular com utilização de células exógenas.

Mais estudos são necessários para delinear completamente os mecanismos responsáveis pelas características imunomoduladoras das células-tronco, além de sua capacidade de movimentação e de integração a tecidos.

Descritores: lesão pulmonar aguda; sepse; transplante de células-tronco.

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INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are devastating disorders of overwhelming pulmonary inflammation that occur in adults and children as a consequence of sepsis, aspiration, trauma, or infection with pathogens, such as coronaviruses (one of which causes severe acute respiratory syndrome), as well as the influenza A viruses H5N1 and H1N1 (causing the avian flu and swine flu, respectively). It is estimated that, in the United States alone, there are 190,600 cases of ALI per year, resulting in 74,500 deaths and 3.6 million hospital days (1). Patients require admission to an intensive care unit and mechanical ventilation (MV), which renders them at risk for ventilator-induced lung injury (VILI) and multiple organ failure. In their weakened state, critically ill patients are more susceptible to ALI, the prevalence of which is therefore quite high in such patients, with devastating consequences (mortality, 50-60%). In addition, for survivors, convalescence is prolonged, the mortality risk remaining high for as long as 5 years after the initial illness (2). Despite advances in supportive care, no specific treatment for ALI exists. The only strategy demonstrated to reduce mortality relies on limiting MV, in order to avoid lung stretch and the consequent VILI (3). Accordingly, there is an urgent, unmet need for new treatments.

Pharmacological approaches to ALI/multiple organ failure have largely focused on suppressing the endogenous inflammatory response, either globally or via inhibition of specific mediators. Despite advances in our understanding of the biology of these related syndromes and the promising results of pre-clinical studies, the basic science has not translated into clinically useful and widely applicable strategies for intervention. The largely disappointing results of clinical trials can be explained in part by our incomplete understanding of the molecular responses to injury, the complications of immunosuppressive therapy, limitations of lung-specific cell delivery systems, the timing of treatment, the role of combination therapy, and the overlapping injurious effects of MV. There is little evidence to suggest that the heterogeneity in outcomes indicates variability in the mechanism of injury or the response to therapy.

Cell-based therapies involving the use of embryonic or adult stem cells, including induced pluripotent stem cells, have emerged as potential novel approaches to a number of devastating and otherwise incurable lung diseases, including emphysema, pulmonary fibrosis, pulmonary hypertension, and ARDS (4-6). The beneficial effects of bone marrow-derived stem cells seem to be related to their immunomodulatory and reparative potential. Mesenchymal stem cells (MSCs), which are immune privileged, have been shown to have therapeutic potential due to characteristics such as plasticity, intrinsic tropism towards lesions, paracrine effects, and immunoregulatory activity. Stem cells are unique cells that have the capacity for self-renewal and differentiation, as well as the ability to take on a specific identity in response to host cells, cytokines, and extracellular components (4-6). In addition, MSCs have been shown to produce large quantities of bioactive factors, which provide molecular cueing for regenerative pathways and affect the status of responding cells intrinsic to the tissue. This environment might accommodate the cells indefinitely, controlling their self-renewal and progeny production in vivo, as well as refining their ability to induce reparative processes in the host. Recent studies have focused on whether the reported beneficial effects of using MSCs are a result of repair and regeneration or simply due to the attenuation of the inflammatory response inherent to cell therapy. In this paper, we review the data in support of the therapeutic use of adult MSCs in the treatment of ALI/ARDS.

Properties of Stem Cells

Stem cells are undifferentiated cells with a capacity for self-renewal and differentiation into multiple cell types. Individual stem cells undergo continuous cell formation, leading to a succession of cells that have a progressively reduced capacity for self-renewal and ultimately becoming lineage-committed cells (7,8).

Stem cells have been broadly classified as adult tissue-derived stem cells and embryonic stem cells. Embryonic stem cells are derived from the inner cell mass of a developing blastocyst and are designated as pluripotent, meaning that they have the ability to differentiate into cells of all embryologic lineages and all adult cell types.

Rippon et al. showed that, in a specific culture medium, embryonic stem cells can give rise to lung progenitor cells with several advantages (9): better cell integration into the host tissue; post-implantation division capacity, which minimises the number of cells that must be transplanted; and a capacity to generate one or more types of adult somatic cells, such as pneumocytes (types I and II) and Clara cells. Although pluripotency gives these stem cells the ability to proliferate indefinitely without differentiation, this characteristic might represent a disadvantage of these cells in that they can undergo uncontrolled growth, leading to formation of neoplasms.

In general, adult MSCs are considered multipotent, having the capacity to differentiate into mature cell types of the parent tissue and a variety of cell lineages (10-12). Although the best-characterised source of MSCs is the bone marrow, these cells can also be isolated from various tissues, such as adipose and lung tissue (13). Because these cells are present in small numbers in the bone marrow, in vitro cell expansion is typically required. In order to define MSCs, the following minimum consensus criteria should be adopted

(14): selection of a plastic-adherent cell population under standard culture conditions; expression of CD105, CD73, and CD90, together with a lack of surface expression of CD45, CD34, CD14, CD11b, CD79–, CD19, and HLA-DR; and the in vitro ability to differentiate into adipocytes, osteocytes, or chondrocytes.

Until recently, the beneficial effects of stem cells were mostly attributed to their ability to incorporate into tissue (engraftment), to differentiate into the appropriate cell type, and to repair injury. Although engraftment is still thought to occur, recent investigations suggest that other mechanisms are involved. It is believed that adult stem cells are recruited to the site of the injury and can exert paracrine effects, generating mediators, such as growth factors, which are necessary for tissue repair (15,16).

In animal models of lung injury, administration of MSCs attenuates the severity of the inflammatory response even when the levels of engraftment are low. Various investigators have reported that MSCs have a remarkable ability to modulate the immune system, including the function and response of dendritic cells, T cells, B cells, and neutrophils (17,18). In addition, from an immunological perspective, allogeneic MSCs are usually well tolerated by the host and have a low immunogenicity pattern because of constitutive low expression of major histocompatibility complex class I and II proteins, as well as, in general, the lack of T-cell costimulatory molecules, such as CD80 and CD86 (19).

Application of MSCs in ALI

According to the American Thoracic Society/European Respiratory Society consensus, ALI is defined as the acute onset of pulmonary infiltrates with severe hypoxemia (20). The pathogenesis of ALI has been associated with direct injury (to the alveolar epithelium) and indirect injury (to the vascular endothelium). The resulting inflammatory changes are accompanied by increased alveolar capillary membrane permeability, which leads to clinical and radiological aspects characteristic of bilateral diffuse airspace disease (ARDS). On CT scans, this can present as: "lobar attenuation" (loss of aeration with no concomitant excess in lung tissue), predominantly in the lower lobes, and "non-lobar attenuation", with diffuse, massive loss of aeration and excess lung tissue in all the pulmonary parenchyma.

Although initial studies using cell-based therapy have suggested engraftment of exogenously administered stem cells in the lung, this is now generally felt to be a rare occurrence of uncertain physiologic significance (21). In addition, the route of administration of donor-derived cells is less well characterised, because most studies have investigated engraftment after systemic administration of donor cells. Direct intratracheal administration of MSCs might enhance retention and promote epithelial engraftment of donor-derived cells in the lung (22). The mechanisms by which circulating or systemically administered stem or progenitor cells are recruited to the lung remain poorly understood. After systemic (i.e., venous) administration, many cells initially migrate to the lungs as the first major capillary bed encountered. Lung injury can result in increased localisation and retention of marrow-derived cells in the lung (23,24).

Little is known about the soluble mediators released by injured lung cells and their role in the intrinsic stem cell tropism for injury, paracrine effects, and immunoregulatory activity. In addition, how circulating cells interact has not been studied in depth. In vitro studies continue to demonstrate that soluble factors released from lung epithelial cells or from injured lung homogenates can induce expression of lung epithelial markers in several types of marrow-derived cells, possibly through activation of beta-catenin signalling pathways (25-27). One novel mechanism of inducing phenotypic change might involve release of membrane-derived microvesicles, a recently recognised means of intercellular communication that involves horizontal transfer of mRNA and proteins between cells (28-30). In studies involving models of lung injury that is more chronic, several well-known pathways, including the SDF-1/CXCR4 axis and CD44 pathways, have been implicated (10,31). In ALI, however, this area is controversial and remains under-explored.

A growing number of studies have demonstrated compelling data on the beneficial effects of MSCs in mouse models of ALI, including acute bacterial pneumonia, endotoxin- or bleomycin-induced lung injury, hyperoxia-induced lung injury, and cecal ligation and puncture-induced sepsis (22,32-36). Systemic and intrapulmonary administration of bone marrow-derived MSCs have both been shown to reduce mortality, improve alveolar fluid clearance, and attenuate inflammation—even when there is minimal or no lung MSC engraftment. Various paracrine mechanisms have been proposed in order to explain the beneficial effects of MSC administration. Such mechanisms include the release of anti-inflammatory mediators such as interleukin-10, angiopoietin-1, and keratinocyte growth factor (5,8,37). Other available animal models of ALI, such as acid-induced ALI and VILI, have been employed in order to evaluate the pathogenesis and pathophysiology of lung injury (38,39). Recent studies involving murine models of lung disease have demonstrated the paracrine effects of stem cell administration, including the stimulation of angiogenesis, together with the modulation of local inflammatory and immune responses. The mechanisms of MSC effects on inflammatory and immune cells are not well understood but likely involve secretion of soluble mediators as well as cell-cell contact (5). Figure 1 shows how MSCs and their paracrine factors can ameliorate or reverse specific injurious changes associated with ALI.

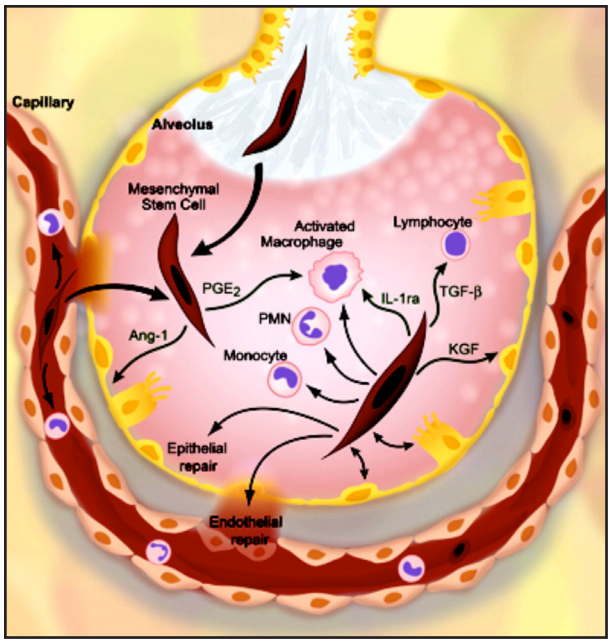

Figure 1 - An injured alveolus with protein-rich oedema fluid and an influx of inflammatory cells, secondary to endothelial as well as epithelial injury, showing the potential for MSCs to be delivered via the air spaces or circulation. Some potential pathways of repair are illustrated: MSC interaction with injured resident alveolar epithelial cells; MSC interaction with injured lung endothelial cells; and MSC modulation of the immune responses of monocytes, polymorphonuclear leukocytes (PMNs), activated macrophages, and lymphocytes, via the secretion of various products, including angiotensin I (Ang-I), prostaglandin E_2 (PGE_2), interleukin-1 receptor antagonist (IL-1ra), transforming growth factor beta (TGF-β), and keratinocyte growth factor (KGF). Several other paracrine factors might also play important roles in reducing lung injury and promoting repair. This figure is reproduced with permission of the publisher (8).

The specific inflammatory environment in the injured lung can influence the MSCs themselves. For example, endotoxin can alter MSC expression of toll-like receptor 4, resulting in increased production of cyclooxygenase-2 and subsequent increased production of the anti-inflammatory agent prostaglandin E2 (35). In endotoxin-treated isolated perfused human lungs, MSCs have been found to have similar anti-inflammatory effects (37). In another interesting study, Zhao et al. showed that MSC protection of lung injury prevented endothelial leakage by strengthening the endothelial adherens junctions through activation of Rho GTPase Cdc42 (40).

The proportion of MSCs among adult bone marrow cells is low (less than 0.1%). However, once isolated, MSCs can be expanded ex vivo, which makes it possible to manufacturer these cells for potential therapeutic purposes. In addition, MSCs can easily be transduced or genetically manipulated to deliver or to secrete selected disease-modifying molecules. When engineered to further express angiopoietin-1 (combination gene and cell-based therapy), MSCs can have a more pronounced protective effect (41). Based on these findings, as well as on safety and initial efficacy data from trials of adult stem cells in other diseases, groundbreaking clinical trials of cell-based therapy for pulmonary hypertension and chronic obstructive pulmonary disease have been initiated. In a clinical trial of MSC administration in chronic obstructive lung disease, the 6-month interim analysis showed a decrease in circulating C-reactive protein and a trend towards improved performance on the six-minute walk test (42). The trial is still in progress, and the long-term follow-up period has yet to be completed. Two other trials demonstrated a positive effect of endothelial progenitor cells on pulmonary hypertension (43,44). A larger clinical trial, using endothelial progenitor cells transduced to express endothelial nitric oxide synthase, was initiated at the University of Toronto (10). The initial data showed a reduction (of nearly 50%) in pulmonary vascular resistance over the 3-day cell delivery period. Novel studies aimed at determining the beneficial effects of adult stem cells in patients with ALI/ARDS are expected to be approved over the next few years. In parallel, the identity and role of endogenous lung progenitor cells in the development and repair of injury continues to be elucidated and could yield information regarding the reparative potential of these cells in ALI.

Summary

Stem cells have shown significant promise in the field of critical care medicine, for prognostication and for treatment strategies. Although there are currently more than 100 MSC-related clinical trials registered in the clinicaltrials.gov database, none are in the field of ALI. Ongoing trials are exploring the viability of MSC administration as a therapeutic option in a wide variety of human diseases, including heart failure, kidney injury, multiple sclerosis, graft vs. host disease, and inflammatory bowel disease. Most of these trials are phase I or phase I/II trials. To date, little has been reported in terms of serious safety concerns arising from these trials. Although the experimental data strongly suggest a potential benefit of MSC-based therapy for patients with ALI, further clinical trials are needed in order to evaluate the mechanisms and pathways of benefit of MSC administration; to test MSC safety and efficacy; and to investigate routes of administration, dosing, and organ-specific cell delivery.

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