

Mesenchymal stem cell therapy in acute lung injury: is it time for a clinical trial?

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Despite decades of research, no specific pharmacological therapy to treat acute lung injury (ALI) has been identified. At present, the only effective therapies act by limiting iatrogenic injury associated with positive fluid balance¹ or mechanical ventilation.^{2,3} As efforts to pharmacologically modulate the complex inflammatory process which leads to alveolar injury have been unsuccessful,⁴ focus has changed to cell based therapy, aimed at utilising stem cells which have pleiotropic effects and which respond appropriately to local signalling molecules.

Stem cells have the capacity for limitless self-renewal and differentiation. Mesenchymal stem cells (MSC) are multipotent adult stem cells with the capacity to differentiate into many different cell types, including alveolar cells. There are several mechanisms through which MSCs could potentially be used for attenuating lung injury and augmenting repair.⁵ On the basis of the currently available data, and supported by two papers,^{6,7} the most important therapeutic effect of exogenously administered MSCs is probably through the paracrine secretion of mediators such as growth factors and cytokines to modulate localised inflammation and tissue repair.⁸ Gupta *et al* found treatment with MSCs improved bacterial clearance in a mouse model of gram-negative pneumonia, which was due in part to lipocalin 2 production by MSCs,⁶ while Curley *et al* showed that MSC therapy improved lung repair via a paracrine mechanism that was keratinocyte growth factor (KGF)-dependent.⁷ However, cell contact-dependent mechanisms, via the gap junction channel protein connexin-43, have also been described.⁹ The other main effect of

exogenously administered MSCs is their localisation to the site of injury and differentiation into mature cells replacing injured cells and promoting repair. Possible further therapeutic approaches include the use of exogenous MSCs as a means of delivering gene therapy or the upregulation of endogenous MSCs via the administration of exogenously administered growth factors.⁵

The preclinical studies address the use of MSCs in ALI and support the need to progress to an early phase clinical trial. As MSCs reduce inflammation, a possible consequence might be reduced host defence with decreased capacity to clear infection. To test the effects of MSCs in ALI due to pneumonia, Gupta and colleagues undertook a series of studies, both *in vitro* and *in vivo*, to investigate the effects and mechanisms of MSCs in a murine model of *Escherichia coli* pneumonia. Intratracheally administered murine MSCs reduced lung injury and improved survival compared with controls. Although an anti-inflammatory effect was seen with MSC administration, in the form of reduced pro-inflammatory cytokine levels, reassuringly microbial clearance was increased. The antimicrobial effect was largely due to the upregulation of lipocalin 2, which acts by sequestering bacterial siderophore-iron complexes, thus limiting bacterial iron supply and contributing a bacteriostatic effect to the innate immune system.¹⁰ This adds to previous work from the same group which found another antimicrobial peptide, LL-37, also contributed to the antibacterial effect of MSCs.¹¹

The reparative potential of MSCs was elegantly demonstrated by Curley *et al* in a novel model of injury and repair following ventilator-induced lung injury.⁷ Postinjury intravenously delivered rat MSCs, or rat MSC conditioned medium (CM), significantly decreased rodent lung injury, as measured functionally, biochemically and pathologically. In an *in vitro* human alveolar epithelial wound repair model, using human MSCs and human MSC CM, again both MSCs and

MSC CM induced greater repair compared with control. Using inhibitors to three growth factors, KGF, hepatocyte growth factor and transforming growth factor β , KGF alone was significantly associated with improved wound repair. Levels of KGF were higher in MSC CM than in fibroblast CM, suggesting that the MSCs were the source of this growth factor.

Given the repeated failure of pharmacological therapies to improve outcome in ALI, a novel approach to ALI research is welcome. Numerous other preclinical studies, ranging from cell to rodent *in vivo* to human *ex vivo* studies, have demonstrated the efficacy of MSCs when used across a spectrum of models of ALI.¹² A recent double blind, randomised controlled trial of MSCs in acute myocardial infarction reported no adverse effects with this therapy and improved functional outcomes.¹³

However, before undertaking human clinical studies, several issues require to be addressed. First, whether it is the MSCs, their secreted products, or both, which are therapeutic in ALI is uncertain. The study by Curley and colleagues⁷ demonstrates that both are therapeutic, with secreted KGF appearing to be the effect mediator. These findings are consistent with data from a human *ex vivo* lung perfusion model of ALI induced by *E coli* endotoxin, where both MSCs and MSC CM were effective in improving alveolar fluid clearance.¹⁴ Consistent with the present study, KGF also appeared to be the crucial mediator of this improvement. KGF has been investigated in a range of experimental lung injury models and in one human model of ALI (ISRCTN98813895). In addition to being a potent mitogen, it has numerous effects including cellular repair, cytoprotection, alveolar fluid clearance modulation and immunomodulation.¹⁵ Potentially, KGF could provide a viable alternative to MSC therapy. A single centre, double blind, randomised controlled trial of KGF in ALI (KARE study, ISRCTN95690673) is ongoing and will further inform this issue.

Second, the antimicrobial effect of MSC therapy requires further thought. The study by Gupta *et al* provides reassuring data in a bacterial model of ALI. This is supported by data from a recent study (published in abstract), from the human *ex vivo* lung perfusion injury model induced by live *E coli*, where MSCs were effective in improving alveolar fluid clearance.¹⁶ The antimicrobial effect of MSC therapy in this current study in *Thorax* was largely due to lipocalin secreted from

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MSCs, rather than MSC CM modifying resident alveolar macrophage activity. It is uncertain how much this antimicrobial effect contributed to the improvement of these injured mice and how much was due to the reparative effects of the MSCs; however, this raises the question as to whether MSCs might be more beneficial for infectious causes of ALI and a product such as KGF be more suitable for non-infectious causes. Additionally, the use of MSCs during catecholamine therapy for septic shock, a group of vasoactive agents known to augment bacterial iron handling,^{17 18} could potentiate their antimicrobial effect given the mechanism of action of lipocalin.

Third, the potential dose regimen remains unknown for ALI. The optimal dose and whether a single dose or multiple dosing regimen is required is unclear. Curley *et al*⁷ demonstrated a beneficial effect with multiple doses. The dosing regimen has varied across studies, as exemplified by these two studies.^{6 7} Also, similarly, the optimal route of administration is unknown; however, both intravenous and intratracheal administration has been used successfully. On balance, supported by these papers, it would seem most appropriate in an initial phase II clinical trial to administer the highest tolerated dose of MSCs intravenously in multiple doses.

Finally, in addition to their potential use during ALI, MSCs or their products could have a role in reconditioning lungs harvested for transplantation, but subsequently deemed too severely injured for use. Ex vivo perfusion of such harvested lungs can restore function to a level suitable for use, and when transplanted, these lungs are not inferior to normally transplanted lungs.¹⁹ The addition of MSCs, or their secreted growth factors, to this ex vivo preparation could have further reparative effects. Approximately 85% of lungs offered for donation are deemed unsuitable due to ALI occurring at the

time of brain death.²⁰ Any intervention which could restore function to these organs and increase transplantation rates is worthy of further investigation.

Rather than attempting to pharmacologically inhibit individual components of the highly conserved, complex homeostatic inflammatory process, MSC therapy affords a new treatment paradigm for ALI. It is time for a clinical trial to answer the questions that the studies by Gupta *et al* and Curley *et al* now compel us to ask: are MSCs a viable therapy for ALI?

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