Biological effects and mechanisms of action of mesenchymal stem cell therapy in chronic obstructive pulmonary disease

Zhixian Jin¹*, Xinghua Pan²*, , Kaihua Zhou¹, Hong Bi¹, Liyan Wang¹, Lu Yu³ and Qing Wang¹

Abstract
Chronic obstructive pulmonary disease (COPD) is the most frequent chronic respiratory disease and a leading cause of morbidity and mortality, worldwide. Given that the foremost risk factor leading to the development of COPD is cigarette smoke, the initial treatment for COPD is smoking cessation. Even after smoking cessation, inflammation, apoptosis and oxidative stress can persist and continue to contribute to COPD. Although current therapies for COPD (which are primarily based on anti-inflammatory drugs such as corticosteroids, theophylline and bronchodilators) reduce airway obstruction, limit COPD exacerbation and improve the patient’s health-related quality-of-life, none can prevent disease progression or reduce mortality. Recent advances in stem cell research have provided novel insight into the potential of bone marrow mesenchymal stem cells (MSCs) in the treatment of several pulmonary diseases. This review article discusses the biological effects and mechanisms of action of MSC transplantation in COPD, and highlights the foundation that MSCs provide for novel therapeutic approaches in COPD.

Keywords
Chronic obstructive pulmonary disease (COPD), emphysema, mesenchymal stem cell (MSC)

Date received: 28 October 2014; accepted: 22 December 2014

¹Second Department of Respiratory Medicine, The First People’s Hospital of Kunming, Kunming, Yunnan Province, China
²Stem Cell Engineering Laboratory of Yunnan Province, Department of Clinical Research, Kunming General Hospital of Chengdu Military Command, Kunming, Yunnan Province, China
³Department of Pathology, The First People’s Hospital of Kunming, Kunming, Yunnan Province, China

*These authors contributed equally to this work.

Corresponding author:
Qing Wang, Second Department of Respiratory Medicine, The First People’s Hospital of Kunming, 504 Qingnian Road, Xishan District, Kunming 650011, Yunnan Province, China.
Email: Wangqing87329@126.com

Creative Commons CC-BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 3.0 License (http://www.creativecommons.org/licenses/by-nc/3.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (http://www.uk.sagepub.com/aboutus/openaccess.htm).
Introduction

The Global Initiative for Chronic Obstructive Lung Disease defines chronic obstructive pulmonary disease (COPD) as a common preventable and treatable disease, characterized by airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. COPD is the most frequent chronic respiratory disease and currently the sixth leading cause of morbidity and mortality worldwide; it is projected to be ranked third by 2020 due to an increase in smoking rates. COPD pathology includes small airway inflammation and emphysema, which is characterized by destruction of alveolar septa, leading to enlargement of the remaining distal airspaces. Although COPD primarily affects the lungs, it also has significant systemic consequences.

Chronic obstructive pulmonary disease is mainly caused by exposure to noxious gases, such as chemicals and air pollutants, with the foremost risk factor being cigarette smoke. Pathogenic mechanisms of cigarette smoke in COPD include chronic inflammation, protease/antiprotease imbalance, apoptosis and oxidative stress. Although the initial treatment for COPD is smoking cessation, inflammation, apoptosis and oxidative stress persist even in the absence of cigarette smoke. Current therapies for COPD (which are primarily based on anti-inflammatory drugs such as corticosteroids, theophylline and bronchodilators) can reduce airway obstruction, limit COPD exacerbation, and improve health-related quality-of-life. None of these therapies prevents COPD progression or reduces mortality, so a pressing need exists for the development of novel COPD therapies.

Recent advances in mesenchymal stem cell (MSC) therapy have made this approach a strong candidate for clinical use in the treatment of several pulmonary diseases. These multipotent non-haematopoietic progenitors can be readily harvested from numerous tissues and expanded with high efficiency, and have strong immunosuppressive properties that can be exploited for successful autologous as well as heterologous transplantations. Transplantation of MSCs has been reported in emphysemic rats, murine asthma, pulmonary hypertension in rats, and acute lung injury in rats. This review article will discuss the biological effects and mechanisms of action of MSC transplantation in COPD.

MSC transplantation may alter inflammatory processes involved in COPD pathogenesis

Chronic inflammation and COPD development

Cigarette smoke has been shown to activate macrophages directly, resulting in the release of oxidants and proteases that mediate alveolar wall destruction and contribute to the establishment of emphysema. Macrophage activation also results in the release of cytokines involved in inflammatory processes in airway and lung parenchyma, including tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. Activated neutrophils have themselves been shown to contribute to alveolar destruction and mucous hypersecretion via the release of oxidants and proteases. Moreover, activated T-cells, in particular CD8+ T-cells, release cytotoxic perforins, granzyme B and TNF-α, which directly cause cell death and apoptosis of alveolar epithelial cells, a feature of emphysema. In addition to activating macrophages,
cigarette smoke has also been shown to activate epithelial cells to secrete a variety of proteases and inflammatory mediators, thereby supporting the inflammatory processes that contribute to emphysema (Figure 1).²³

**Inhibition of inflammation by transplantation of MSCs**

One of the mechanisms postulated for MSC protection against emphysema is suppression of the inflammatory response by modulating the release of soluble anti-inflammatory molecules and activation of cellular anti-inflammatory pathways.²⁴ Intrapulmonary administration of MSCs in a rat model of cigarette smoke-induced emphysema has been shown to ameliorate emphysematous pathology in these animals, in part via downregulation of proinflammatory mediators such as TNF-α, IL-1β, IL-6 and MCP-1.²⁴ Moreover, intravenous infusions of allogeneic MSCs suppressed levels of circulating C-reactive protein in a clinical trial of patients with COPD, although no significant differences in pulmonary function testing or frequency of COPD exacerbations were noted between MSC-treated patients and controls.²⁵ Contradictions between these two studies concerning the effect of MSCs on pulmonary function may be attributed to differences in dosing and treatment, lack of correlation between rodent models and clinical disease, and the small sample size in the clinical trial.²⁴,²⁵ Further larger scale trials will be necessary, to examine the potential effects of MSCs on clinical outcome in patients with COPD in greater detail.

**Figure 1.** Schematic diagram summarizing the cigarette smoke-induced pathogenic processes involved in the development of chronic obstructive pulmonary disease (COPD). VEGF, vascular endothelial growth factor; NF-κB, nuclear factor-κB; ECM, extracellular matrix.
MSC transplantation and regulation of protease/antiprotease imbalance in COPD pathogenesis

Protease/antiprotease imbalance in COPD

A delicate balance between protease and antiprotease activity is required for proper lung maintenance; derangement of this can result in increased alveolar destruction and the development of emphysema. The inflammatory and oxidative processes associated with exposure to cigarette smoke have been shown to increase protease production and release from inflammatory and structural cells, and to reduce the activity of antiproteases such as α1-antitrypsin. The resulting protease/antiprotease imbalance contributes to alveolar wall destruction and airspace enlargement via degradation of the extracellular matrix, promoting apoptosis of structural cells in the alveolar walls, and increasing mucus hypersecretion (Figure 1).

Inhibition of protease release by MSC transplantation

Pulmonary administration of MSCs has been shown to reverse the induction of matrix metalloproteinase (MMP)-9 and MMP-12 in the lungs of rats with cigarette smoke-induced emphysema, at both the mRNA and protein levels. Although the mechanistic basis of this effect is not completely understood, it has been attributed in part to the inhibition (by MSCs) of a positive feedback loop, involving the release of proteases by inflammatory and structural cells activated by cigarette smoke, and the recruitment by these proteases of additional inflammatory cells.

Suppression of alveolar apoptosis by MSC transplantation in COPD

Apoptosis and COPD

Apoptosis is a tightly regulated form of cell death that is critical for the maintenance of normal tissue homeostasis and which, under normal conditions, is in equilibrium with cell proliferation. Apoptosis of alveolar epithelial cells is known to play a pivotal role in the pathogenesis of emphysema. It has been shown that increased apoptosis of alveolar epithelial cells in patients with COPD is not balanced by an increase in their proliferation, a state that may contribute to a disturbance in COPD of the steady state balance between apoptosis and proliferation in lung tissue (Figure 1).

Inhibition of alveolar cell apoptosis by MSC transplantation

Blocking the vascular endothelial growth factor (VEGF) signalling pathway leads to apoptosis of the alveolar cell; and decreases in VEGF and VEGF receptor 2 (VEGFR2) at both the protein and mRNA levels have been described in emphysematous patients and smokers. Given that MSCs stimulate VEGF secretion and VEGFR2 induction, amelioration by MSC transplantation of alveolar cell apoptosis in the lungs of papain- or cigarette smoke-induced emphysematous rat models of emphysema has therefore been postulated to involve reversal of the effects of cigarette smoke exposure on the VEGF signalling pathway.

An alternative mechanism by which MSCs suppress alveolar cell apoptosis has been suggested to involve alterations in the expression of apoptotic or antiapoptotic genes in these cells. It has been reported, for example, that the apoptotic gene Bax and the antiapoptotic gene Bcl-2 are induced and repressed, respectively, after pulmonary administration of MSCs in a papain-induced model of emphysema in rats. A third mechanism for MSC amelioration of alveolar apoptosis is suggested by the suppression by MSCs of alveolar levels of cleaved-caspase 3, a key player in the apoptotic programme in these cells.
MSC transplantation may alter levels of oxidative stress in COPD

Oxidative stress and COPD development

Oxidants contributing to the pathogenesis of COPD may originate endogenously, by metabolic reactions, or exogenously, primarily in the form of cigarette smoke. The release of oxidants by inflammatory cells activated by cigarette smoke further increases the oxidative burden imposed by exogenous oxidants. Under normal conditions, a robust pulmonary extra- and intracellular antioxidant defence system protects lung cells from oxidant damage by maintaining a balance between oxidants and antioxidants. A shift in this balance associated with exposure to cigarette smoke, resulting either from an excess of oxidants or depletion of antioxidants, is referred to as oxidative stress, and has been suggested as a pathogenic mechanism in patients with COPD (Figure 1).

The contribution of oxidative stress to COPD is thought to encompass a variety of mechanisms. For example, oxidative stress has been suggested to enhance lung inflammation via induction of redox-sensitive inflammatory transcription factors such as nuclear factor-κB (NF-κB) and activating protein-1 (AP-1), and subsequent stimulation of their cognate transcriptional programmes. In addition, oxidative stress has been shown to increase neutrophil sequestration in the lung, enhancing lung inflammation and establishing a vicious cycle of increased oxidative stress and enhanced inflammation. Oxidative stress has also been associated with protease/antiprotease imbalance and increased alveolar cell apoptosis by inhibition of the VEGF receptor. It has also been linked with direct DNA damage, stimulation of the release of mucus by airway epithelial cells, and impaired mucociliary clearance.

Inhibition of oxidative stress by MSC transplantation

Modulation by MSCs of the redox environment is a rapidly emerging area of interest. For example, the increased survival rate of lipopolysaccharide-induced lung injury rats after transplantation of bone marrow MSCs has been shown to be accompanied by decreased oxidative stress. Moreover, reduction by MSCs of pulmonary levels of malondialdehyde occurs in parallel with increased synthesis of heme oxygenase-1, an enzyme with strong antioxidative stress and cytoprotective effects. In addition, transplantation of bone marrow MSCs is known to decrease oxidative stress in the brain of a rat model of spontaneous stroke, suggesting that MSCs may decrease oxidative stress in cigarette smoke-induced emphysema. Further studies are needed to understand the effects of MSCs on oxidative stress in emphysema, and the antioxidative mechanism of its action in alveolar cells.

Alveolar differentiative potential of transplanted MSCs in COPD

The beneficial role of transplanted MSCs in emphysema has been attributed in part to differentiation of MSCs into alveolar cells, although the exact type of cell is an area of controversy. Differentiation of MSCs into type I and/or type II alveolar epithelial cells has been reported in rat models of lipopolysaccharide- and cigarette smoke-induced emphysema and bleomycin-induced lung injury. On a mechanistic level, Liu et al. have shown that differentiation of MSCs into type II alveolar epithelial cells in a coculture system was associated with activation of the canonical Wnt signalling pathway.

Conclusions

At present, there are no therapies that can reduce the disease progression or mortality
associated with COPD. Transplantation of MSCs represents a potentially promising therapy for COPD, and may involve modulation of inflammation, protease/antiprotease balance, apoptosis and oxidative stress, or the differentiation of MSCs into lung parenchyma cells. A major obstacle to the clinical application of MSCs in COPD is the dearth of data on the long-term safety of MSCs in patients with COPD. It should be noted, however, that a clinical trial has shown no infusional toxicity, serious adverse events, or attributable deaths in MSC-treated patients during a 2-year follow-up period. Further larger scale clinical trials will be necessary, to more fully assess the efficacy and long-term safety of MSCs in patients with COPD. A second major challenge to the clinical application of MSCs in COPD is that the therapeutic schedule is not clear, and additional studies are warranted to ascertain the appropriate cellular dose, infusion rate and route of administration. A final challenge is the poor survival of MSCs and the low level of engraftment in host organs, therefore, a pressing need exists for the development of approaches that increase survival and engraftment of MSCs in host organs. In summary, although several challenges exist, transplantation of MSCs represents a potentially promising therapy for COPD.

**Declaration of conflicting interest**

The authors declare that there are no conflicts of interest.

**Funding**

The development of this review was supported by a grant from the Applied Basic Research Project Fund of Yunnan Province, China (grant no. 2012FB106).

**References**

1. Vestbo J, Hurd SS, Agustí AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2013; 187: 347–365.
2. Vaz Fragoso CA and Gill TM. Defining chronic obstructive pulmonary disease in an aging population. *J Am Geriatr Soc* 2010; 58: 2224–2226.
3. The definition of emphysema. Report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. *Am Rev Respir Dis* 1985; 132: 182–185.
4. Celli BR and MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004; 23: 932–946.
5. Marlow SP and Stoller JK. Smoking cessation. *Respir Care* 2003; 48: 1238–1254.
6. Tuder RM, McGrath S and Neptune E. The pathobiological mechanisms of emphysema models: what do they have in common? *Pulm Pharmacol Ther* 2003; 16: 67–78.
7. Demedts IK, Demoor T, Bracke KR, et al. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respir Res* 2006; 7: 53.
8. Arja C, Surapaneni KM, Raya P, et al. Oxidative stress and antioxidant enzyme activity in South Indian male smokers with chronic obstructive pulmonary disease. *Respirology* 2013; 18: 1069–1075.
9. Siedlinski M, Postma DS, van Diemen CC, et al. Lung function loss, smoking, vitamin C intake, and polymorphisms of the glutamate-cysteine ligase genes. *Am J Respir Crit Care Med* 2008; 178: 13–19.
10. Gamble E, Grootendorst DC, Hattotuwa K, et al. Airway mucosal inflammation in COPD is similar in smokers and ex-smokers: a pooled analysis. *Eur Respir J* 2007; 30: 467–471.
11. Hodge S, Hodge G, Holmes M, et al. Increased airway epithelial and T-cell apoptosis in COPD remains despite smoking cessation. *Eur Respir J* 2005; 25: 447–454.
12. Louhelainen N, Rytilä P, Haaheta T, et al. Persistence of oxidant and protease burden in the airways after smoking cessation. *BMC Pulm Med* 2009; 9: 25.
13. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Pulmonary Disease.
14. Zhao Y, Xu A, Xu Q, et al. Bone marrow mesenchymal stem cell transplantation for treatment of emphysematous rats. *Int J Clin Exp Med* 2014; 7: 968–972.

15. Cho KS, Park MK, Kang SA, et al. Adipose-derived stem cells ameliorate allergic airway inflammation by inducing regulatory T cells in a mouse model of asthma. *Mediators Inflamm* 2014; 2014: 436476. doi: 10.1155/2014/436476. Epub 2014 Aug 26.

16. Tian HJ, Yang JP and Wang XX. The effect of bone marrow mesenchymal stem cell transplantation on hypoxic pulmonary hypertension in rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 2014; 30: 233–236. (in Chinese, English Abstract).

17. Liu QP, Zhou DX, Sun L, et al. Bone marrow mesenchymal stem cells ameliorates seawater-exposure-induced acute lung injury by inhibiting autophagy in lung tissue. *Patholog Res Int* 2014; 2014: 104962. doi: 10.1155/2014/104962. Epub 2014 Aug 19.

18. Le Blanc K and Pittenger M. Mesenchymal stem cells: progress toward promise. *Cytotherapy* 2005; 7: 36–45.

19. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 160(5 Pt 2): S29–S32.

20. Corhay JL, Henket M, Nguyen D, et al. Leukotriene B4 contributes to exhaled breath condensate and sputum neutrophil chemotaxis in COPD. *Chest* 2009; 136: 1047–1054.

21. Bartoli ML, Di Franco A, Vagaggini B, et al. Biological markers in induced sputum of patients with different phenotypes of chronic airway obstruction. *Respiration* 2009; 77: 265–272.

22. Hashimoto S, Kobayashi A, Kooguchi K, et al. Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2000; 161: 237–243.

23. Yagi O, Aoshiba K and Nagai A. Activation of nuclear factor-kappaB in airway epithelial cells in patients with chronic obstructive pulmonary disease. *Respiration* 2006; 73: 610–616.

24. Guan XJ, Song L, Han FF, et al. Mesenchymal stem cells protect cigarette smoke-damaged lung and pulmonary function partly via VEGF-VEGF receptors. *J Cell Biochem* 2013; 114: 323–335.

25. Weiss DJ, Casaburi R, Flannery R, et al. A placebo-controlled, randomized trial of mesenchymal stem cells in COPD. *Chest* 2013; 143: 1590–1598.

26. Mercer BA, Kolesnikova N, Sonett J, et al. Extracellular regulated kinase/mitogen activated protein kinase is up-regulated in pulmonary emphysema and mediates matrix metalloproteinase-1 induction by cigarette smoke. *J Biol Chem* 2004; 279: 17690–17696.

27. Churg A, Wang RD, Tai H, et al. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. *Am J Respir Crit Care Med* 2003; 167: 1083–1089.

28. Yokohori N, Aoshiba K and Nagai A. Increased levels of cell death and proliferation in alveolar wall cells in patients with pulmonary emphysema. *Chest* 2004; 125: 626–632.

29. Kanazawa H and Yoshikawa J. Elevated oxidative stress and reciprocal reduction of vascular endothelial growth factor levels with severity of COPD. *Chest* 2005; 128: 3191–3197.

30. Wong AP, Dutly AE, Sacher A, et al. Targeted cell replacement with bone marrow cells for airway epithelial regeneration. *Am J Physiol Lung Cell Mol Physiol* 2007; 293: L740–L752.

31. Zhen G, Xue Z, Zhao J, et al. Mesenchymal stem cell transplantation increases expression of vascular endothelial growth factor factor in papain-induced emphysematous lungs and inhibits apoptosis of lung cells. *Cytotherapy* 2010; 12: 605–614.

32. Zhen G, Liu H, Gu N, et al. Mesenchymal stem cells transplantation protects against rat pulmonary emphysema. *Front Biosci* 2008; 13: 3415–3422.

33. Kim SY, Lee JH, Kim HJ, et al. Mesenchymal stem cell-conditioned media recovers lung fibroblasts from cigarette
smoke-induced damage. *Am J Physiol Lung Cell Mol Physiol* 2012; 302: L891–L908.

34. Pryor WA and Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann N Y Acad Sci* 1993; 686: 12–27.

35. Morrison D, Rahman I, Lannan S, et al. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. *Am J Respir Crit Care Med* 1999; 159: 473–479.

36. MacNee W. Oxidants/antioxidants and chronic obstructive pulmonary disease: pathogenesis to therapy. *Novartis Found Symp* 2001; 234: 169–185.

37. Pantano C, Reynaert NL, van der Vliet A, et al. Redox-sensitive kinases of the nuclear factor-kappaB signaling pathway. *Antioxid Redox Signal* 2006; 8: 1791–1806.

38. Lehr HA, Kress E, Menger MD, et al. Cigarette smoke elicits leukocyte adhesion to endothelium in hamsters: inhibition by CuZn-SOD. *Free Radic Biol Med* 1993; 14: 573–581.

39. Carp H and Janoff A. Possible mechanisms of emphysema in smokers. In vitro suppression of serum elastase-inhibitory capacity by fresh cigarette smoke and its prevention by antioxidants. *Am Rev Respir Dis* 1978; 118: 617–621.

40. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000; 106: 1311–1319.

41. Takeyama K, Dabbagh K, Jeong Shim J, et al. Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. *J Immunol* 2000; 164: 1546–1552.

42. Li J, Li D, Liu X, et al. Human umbilical cord mesenchymal stem cells reduce systemic inflammation and attenuate LPS-induced acute lung injury in rats. *J Inflamm (Lond)* 2012; 9: 33.

43. Fredenburgh LE, Perrella MA and Mitsialis SA. The role of heme oxygenase-1 in pulmonary disease. *Am J Respir Cell Mol Biol* 2007; 36: 158–165.

44. Calió ML, Marinho DS, Ko GM, et al. Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model. *Free Radic Biol Med* 2014; 70: 141–154.

45. Rojas M, Xu J, Woods CR, et al. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005; 33: 145–152.

46. Kotton DN, Ma BY, Cardoso WV, et al. Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development* 2001; 128: 5181–5188.

47. Huang K, Wu XM, Wang XY, et al. The effect of marrow mesenchymal stem cell transplantation on pulmonary fibrosis in rats. *Zhonghua Jie He He Hu Xi Za Zhi* 2012; 35: 659–664. (in Chinese, English Abstract).

48. Liu AR, Liu L, Chen S, et al. Activation of canonical wnt pathway promotes differentiation of mouse bone marrow-derived MSCs into type II alveolar epithelial cells, confers resistance to oxidative stress, and promotes their migration to injured lung tissue in vitro. *J Cell Physiol* 2013; 228: 1270–1283.

49. Parekkadan B and Milwid JM. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng* 2010; 12: 87–117.