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Are mesenchymal stem cells a chance for the breakthrough in the treatment of acute respiratory distress syndrome?

Czy mezenchymalne komórki macierzyste są szansą na przełom w leczeniu ARDS?

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S u m m a r y

Acute respiratory distress syndrome (ARDS) is a clinical condition relating to a group of severe and acute lung injuries of different etiology. The syndrome is characterized by an extensive local lung inflammation leading to the destruction of the endothelial-epithelial barrier, neutrophils infiltration, formation of hyaline membranes, edema, reduction of compliance and hypoxia. High mortality rates and the failures of the clinical trials up to date with potential drugs encourages an intensive search for a new innovative pleiotropic therapeutic regimen. Mesenchymal stem cells (MSCs) constitute a population of cells with the ability to differentiate into lineages of the connective tissue and also possess the capacity to modulate both innate and adaptive immune response. These features of MSCs have justified their experimental use in the models of ARDS. In the last years it has been shown in different models that the administration of MSCs can reduce the inflammatory response, decrease lung injury but enhance the antibacterial regimens, altogether leading to the reduction of mortality. In this article we present the features of MSCs that may have an impact on the course of ARDS and we review the preclinical trials of MSCs in this syndrome. We conclude by discussing potential difficulties and challenges that can arise during the introduction of MSCs into the clinical treatment of ARDS.

S t r e s z c z e n i e

Zespół ostrej niewydolności oddechowej (ang. *acute respiratory distress syndrome* – ARDS) jest stanem klinicznym obejmującym ciężkie i ostre uszkodzenie płuc o różnej etiologii. Zespół ten charakteryzuje się nadmierną reakcją zapalną w płucach prowadzącą do niszczenia bariery śródbłonkowo-nabłonkowej, powstania nacieków neutrofilowych, błon hialinowych, obrzęku i zmniejszenia podatności płuc i hipoksji. Wysoka śmiertelność i niepowodzenia dotychczasowych prób klinicznych z wykorzystaniem potencjalnych leków skłaniają do poszukiwania nowych opcji terapeutycznych działających wielokierunkowo. Spore nadzieje w tej mierze wiąże się z mezenchymalnymi komórkami macierzystymi (ang. *mesenchymal stem cells* – MSCs). Komórki te, występujące w dorosłym organizmie, mają zdolność do różnicowania się w dojrzałe komórki tkanki łącznej, a także do modulowania wrodzonej i nabytej odpowiedzi immunologicznej. Ze względu na te właściwości mogą się okazać przydatne w leczeniu ARDS. W ostatnich latach wykazano na podstawie badań różnych modeli ARDS, że podanie macierzystych komórek może wyciszać reakcję zapalną, redukować uszkodzenie płuc, a jednocześnie poprawiać zdolność do zwalczania infekcji, co razem powoduje zmniejszenie śmiertelności zwierząt z ARDS. W poniższym artykule przedstawiamy właściwości MSCs mogące mieć znaczenie terapeutyczne w odniesieniu do ARDS, a także dokonujemy przeglądu badań przedklinicznych wykorzystujących MSCs w leczeniu tego zespołu. Ostatecznie rozważamy również potencjalne trudności, jakie może napotkać wprowadzenie MSCs do praktyki klinicznej.

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Abbreviations:
PaO_2 – Partial pressure of oxygen in the arterial blood, FiO_2 – Fraction of inspired oxygen, PaO_2/FiO_2 – Index of oxygenation, TNF – Tumor necrosis factor, IL – Interleukin, CD – Cluster of differentiation, PGE – Prostaglandin, TGFβ – Transforming, growth factor beta, BALf – Bronchoalveolar lavage fluid

ARDS – CONSTANT CHALLANGE

Acute respiratory distress syndrome (ARDS) is the most severe form of lung injury with mortality reaching up to 40% (1). It is also one of the most common clinical syndromes treated in the intensive care units (ICU). The etiology of ARDS is very broad and associated with multiple other conditions. Clasically, two sources of ARDS are distinguished: pulmonary (i.e. pneumonia, choking, inhalatory burn) and non-pulmonary sources (sepsis, acute pancreatitis, trauma, massive blood transfusions). The most frequent cause of ARDS is sepsis (2). The diagnostic proces is based on clinical signs like: acute beginning, hypoxia ($PaO_2/FiO_2 < 300$), bilateral opacities on chest imaging not explained by other pulmonary pathology (e.g. pleural effusion, pneumothorax, or nodules) and respiratory failure not explained by heart failure or volume overload (3). Heterogenous clinical picture and very wide range of patients together with lack of specific symptoms constitute the first therapeutical problem. Although the pathomorphological changes in ARDS are relatively well defined (presence of hyalin membranes, neutrophil infiltrates, significant alveolar damage followed by a pattern of fibrosis and regeneration), the pathophysiological processes are complicated and remain not well understood. The first, acute phase of ARDS is characterized by exaggerated inflammatory response reflected by e.g. increased levels of pro-inflammatory cytokines: TNF, IL-6, IL-8 in serum and bronchoalveolar lavage fluid, activation of pulmonary macrophages and infiltration of activated neutrophils. These processes along with factors inducing ARDS (e.g. bacterial toxins, hot gases) evoke apoptosis of pneumocytes and injury of the microcirculatory endothelium of lungs. The injury of endothelial-epithelial barrier enhances further influx of neutrophils and is a cause of pulmonary edema (4, 5). Even in the early phase of the disease, simultaneously with the inflammatory process, activation of fibroblasts and regenerative mechanisms begins (6). Nowadays, patients rarely die because of the early respiratory failure. Common cause of deaths include nosocomial infections and development of Multiorgan failure. In 50% of patients who died with ARDS, lung fibrosis is observed, what suggests impaired activation of regenerative mechanisms what causes deterioration of clinical state (7).

In spite of many clinical trials and many years of intensive research, there is no effective treatment of ARDS so far. Conducted trials with glucocorticoids, exogenous surfactant, inhaled nitric oxide, prostaglandins, anticoagulants did not improve the outcome (8). Reduction of mortality was only achieved by introducing protective mechanical ventilation (airway pressure not exceeding 30 cm H_2O), restrictive fluid therapy and ventilation in prone position (9-11). These procedures rather limit the iatrogenic injury than treat the disease. Due to the complex pathophysiology and harmful influence of injured lungs on other organs, effective treatment of ARDS should be multidirectional.

MESENCHYMAL STEM CELLS – UNIQUE FEATURES PAVE THE WAY

Originally, mesenchymal stromal cells (called also mesenchymal stem cells) have been isolated and characterized from the bone marrow by Friedenstein et al. (12). These multipotent stem cells have the capacity to multilineage differentiation into the cells of the connective tissue and therefore play an important role in the regenerative mechanisms of the adult organism. Other research groups have identified these cells in virtually every organ of the human body (lungs, heart, liver, adipose tissue, placenta, cord blood) (13). Localization among the pericytes surrounding vessels present in all tissues explain nicely the widespread presence of these cells in the body (14). Primary and still the most important method of isolation of MSCs is a culture of adherent cell colonies from a given tissue. Although, a consensus was made about the methods that confirm presence of MSCs, there is a lack of efficient method of their isolation based on one antigen. Such method is needed to obtain a homogenous population of cells. Criteria of MSCs are fulfilled by cells that are able to form adherent colonies in the *in vitro* culture, under special medium can differentiate into osteoblasts, adipocytes and chondrocytes and finally they express surface markers as: CD90, CD105, CD73, CD44 but not: CD45, CD34, CD31 (15).

MSCs are in the focus of interest of investigators working on the new therapies of multiple diseases (e.g. graft versus host disease, inflammatory bowel disease, infarction, multiple sclerosis) (16). MSCs seem so attractive because of their capacities to modulate the immune response and injured tissues, although their potential to differentiate into harmed tissues is also important. First reports on the utility of MSCs in the regeneration of injured lungs, suggesting high level of engraftment by transplanted cells (17), were not confirmed by other groups (18-21). In these reports, the level of engraftment in the injured lungs was below 1% of lung cells what suggests other mechanisms of action of injected MSCs. Currently, many research are aimed at investigating the immunomodulatory properties of MSCs. Numerous papers reported immunosuppressive effects of these cell on both innate and adaptive immunity (22-25). This effect is achieved by direct contact between cells and also by paracrine mediators. MSCs produce factors like: PGE_2, indoleamine 2,3-dioxygenase, cytokines: TGFβ, IL-1RA, IL-10. The role of PGE_2 was widely investigated and described: produced by MSCs – "re-programs" pulmonary macrophages during sepsis to produce IL-10 (26). IL-10 is a key mediator in the protective pathways utilized by transplanted MSCs in the organ injury models in sepsis (26). In the animal model of direct lung injury by endotoxin with subsequent intratracheal application of MSCs, the reduction of mortality and improvement of pathomorphological picture of lungs was correlated with reduced concentration of TNF and increased level of IL-10 in the BALF and serum (27). However,

the interaction between MSCs and immunity is more complexed. These cells can also improve the anti-microbial defense what is extremely important in the sepsis induced ARDS and in the prevention of nosocomial infections in sterile ARDS. After stimulation with TNF, MSCs secrete IL-6 (28), which was shown in *in vitro* models to induce production of IgG by B cells (29). Also, an effect of MSCs on granulocytes is very interesting. MSCs inhibit apoptosis and degranulation of granulocytes, improving their phagocytic capacity (30). MSCs also secrete anti-bacterial peptide called LL-37 (31), what makes themselves a part of the immune system. Abovementioned interactions seem however, quite selective knowing the results of studies examining effect of MSCs on the pattern of genes expression in a murine model of sepsis. The study has revealed that systemic application of MSCs re-programs expression of hundreds of immune-related genes (32).

Aside from immunomodulation, MSCs can also positively affect ARDS by interaction with pneumocytes and endothelial cells building the capillary-alveolar barrier. Growth factors secreted by MSCs (like keratinocyte growth factor – KGF) can limit the injury of parenchyma in the model of lung injury induced by chloric acid or bleomycin (33, 34). KGF protects epithelial cells and also up-regulates expression of sodium pump and increase activity of Na-K ATPase (35), enabling resorption of alveolar fluid. MSCs can also influence the endothelial cells of lung microvessels by KGF and hepatocyte growth factor (HGF) which stabilize endothelial layer in a few mechanisms (36).

Widespread use of MSCs in medicine is possible due to their low immunogenicity. Lack of expression of the major histocompability complex II (MHC II) and low expression of the major histocompability complex I by MSCs cause that these cells are not recognized by the donor's CD4 lymphocytes what enables their allogenic transplantation without previous antigenic match (37).

ANIMAL MODELS – A LIGHT IN THE TUNEL

Kotton et al. have shown that mesenchymal stromal cells have the ability to engraft bleomycin-injured lungs in much higher frequency than healthy lungs (18). These experiments inspired further experimental trials with MSCs in lung injury models. Application of bleomycin induces early inflammatory response followed by chronic fibrosis similar to this observed during idiopathic lung fibrosis in human. More relevant model of human ARDS can be achieved by inducing experimental sepsis by intratracheal or systemic infusion of endotoxin or by surgical procedure called caecum ligation and puncture (CLP). One more relevant model reflecting clinical ARDS is ventilator induced lung injury (VILI), induced by mechanical ventilation with big inspiratory volumes (38). All these models were utilized in studies with mesenchymal stem cells. A brief review of most important studies is summarized in the table 1. Studies that were carried out varied with the source of transplanted cells. Most common source of MSCs was allogenic murine cells from bone marrow, also human fetal cells (cord blood, amnion), bone marrow and adipose-derived cells were investigated. Cells were applied by routes like intravenous and intratracheal way. The so far, the obtained results were similar, however there is a lack of studies comparing efficacy of different application routes. All the published studies reported reduced mortality, increased functionality and protection of the lung structure with normalization of inflammatory (tab. 1).

In sepsis models of CLP, authors observed reduction of bacteremia and enhanced function of immunocompetent cells, what was already discussed above. There is also a noteworthy paper describing model of of human lung perfused *ex vivo* with medium supplemented with fresh blood and injured by intrabronchial application of endotoxin (39). In this model, accumulation of alveolar edema, inflammatory infiltrations and increased concentration of a IL-1β, TNF and IL-8 were seen. Addition of allogeneic MSCs to the perfusing medium in one hour after the application of endotoxin resulted in resorbtion of edema, decrease in pro-inflammatory cytokines and reduction of inflammatory infiltrates. Authors of this work hypothesized that KGF can be the secreted factor responsible for the observed effects of MSCs. This hypothesis was proved by application of KGF alone in the same experimental model. Also use of MSCs with silenced expression of KGF significantly reduced the therapeutical effect. This study elegantly presents potent application of MSCs in the treatment of ARDS in the patients.

CLINICAL POINT OF VIEW – CONCLUSIONS

Progress in the field of regenerative medicine is tremendous. The number of new innovative application of stem cell based therapies steadily increases. This trend is also reflected by the growing number of clinical applications of stem cells. The clinical trial database www.clinicaltrials.gov shows more than 370 registered clinical trials in various diseases after search of 'mesenchymal stem cells' (January 2014). So far, no serious side effects after application of MSCs were reported. Taking this fact into consideration together with the results of above discussed experimental and pre-clinical studies, it seems that first clinical trials with MSCs in the treatment of ARDS will be undertaken soon. However, some critical and ambiguous issues should be discussed.

The first issue is not fully understood mechanism of action of MSCs. First papers reported engraftment of injected MSCs and their differentiation into mature pneumocyte I (18), in the later studies, the paracrine effect on the host tissues was highlighted. Other important aspect is the role of endogenous MSCs during ARDS which is completely not known. It should be assumed that the tissue resident MSCs may play harmful role in the pathogenesis of ARDS. It was shown that MSCs in the parotid gland can *in vitro* enhance the chemotaxis of neutrophils (47) that could by an unwanted process in the non-infectious ARDS.

Table 1. Review of selected papers reporting application of MSCs in the treatment of experimental lung injury. If not stated, allogenic cells were used.

Cell source (cell number) – application	Model	Main effects	Authors
BM-MSCs (1 – 2 x 10^6) – IV	mouse; IT-bleomycin-MSCs after 5 days	– engraftment in injured lung – differentiation in pneumocytes I	Kotton et al. 2001 (18)
BM-MSCs (5 x 10^5) – IV	mouse; IT-bleomycin-MSCs after 6 hours	– differentiation in pneumocytes – inhibition of fibrosis	Rojas et al. 2005 (40)
BM-MSCs (5 x 10^5) – IV	mouse; IP-endotoxin-MSCs after 1 hour	↓ infiltration ↓ edema ↓ INF-γ, IL-1, KC, MIP-1	Xu et al. 2008 (41)
BM-MSCs (7.5 x 10^5) – IT	mouse; IT-endotoxin-MSC after 4 hours	↑ survival ↓ edema ↓ TNF, MIP-2 in BALF and serum	Gupta et al. 2007 (27)
hMSCs umbilical cord (10^6) – IV	mouse; IT-bleomycin-MSCs after 24 hours	– lack of differentiation – accumulation in the injured focci ↓ TGF-β, INF-γ, MIF ↓ collagen and fibrosis	Moodley et al. 2009 (42)
BM-MSCs (2.5 x 10^5) – IV	mouse; CLP-MSCs after 6 hours	↓ mortality ↓ alveolar protein concentration ↓ inflammatory infiltration ↓ kindey and live injury ↓ pro- and anti-inflammatory cytokines ↑ antibacterial defense – change in expression of numerous genes related with inflammation	Mei et al. 2010 (32)
hBM-MSCs (2.5 x 10^5) – IT and IP	mouse; IT-endotoxin-MSCs after 4 hours	↓ neutrophil infilitration ↓ edema ↓ alveolar protein concentration ↓ MIP-2, IL-6, IL-17, MCP-1, G-CSF	Danchuk et al. 2011 (43)
hAD-MSCs (3 x 10^5) – IV	mouse; IT-endotoxin-MSCs after 20 min.	↓ alveolar protein concentration ↓ neutrophil infilitration ↓ iNOS, TGF-β, MIP, IL-12	Chien et al. 2012 (44)
BM-MSCs (2 x 10^6) – IV	rat; VILI-MSCs after 24 hours	↑ blood saturation ↑ lung compliance ↓ extravascular lung water ↓ lung injury	Curley et al. 2012 (45)
hMSCs from cord blood (5 x 10^5) – IV	rat; IP-endotoxin-MSCs after 1 hour	↓ mortality ↓ TNF, IL-1β, IL-6, in serum – no change in IL-10 ↓ inflammation ↓ edema ↑ heme oxygenase	Li et al. 2012 (46)

BM-MSCs – bone marrow-derived MSCs, hMSCs – human MSCs, hAD-MSCs – adipose-derived MSCs, IT – intratracheal, IV – intravenously, IP – intraperitoneal, BALF – bronchoalveolar lavage fluid, INF-γ – Interferon-γ, IL – interleukin, KC – keratinocyte-derived chemokine, MIP-1 – macrophage inflammatory protein-1, TNF – tumor necrosis factor, MIP-2 – macrophage inflammatory protein-2, TGF-β – tranforming growth factor-β, MIF – macrophage migration inhibitory factor, MCP-1 – monocyte chemoattractant protein-1, G-CSF – granulocyte colony-stimulating factor, iNOS – inducible nitric oxide synthase

Next major problem is a translation of the results from animal studies into the clinical setting. The animal model studies are usually performed in the short time (up to several hours) after the initiation of lung injury. In the clinical setting it is not possible to introduce a cell based therapy in such short period of time, especially when the diagnosis of ARDS is often delayed. A patient who is admitted to the intensive care unit often already has developed ARDS and the efficacy of MSCs in such phase of disease was not investigated. The degree of injury caused by systemic comorbidities like sepsis, acute pancreatitis and Multiorgan failure can influence the efficacy and the outcome of MSCs therapy. It seems that the therapy with MSCs should not be applied in the same way in patients with ARDS of different etiology. Moreover, there is a discussion on the route of administration of MSCs. Currently, two routes are commonly investigated: intravenous and intratracheal. MSCs administered intratracheally *a priori* localize in the lungs, while administered intravenously migrate to the injured organ. The impact of source of administered MSCs is also of great significance. Whether the autologous cells taken from the patient`s adipose tissue or bone marrow would be effective and their number enough? Maybe the allogeneic cells from cord blood or amnion would be more feasible? The dose of the administered cells used in the cited papers is around 10^5 – 2 x 10^6 per 25 g of mouse body. For the human, after multiplication of the body mass such dose should be around 3 x 10^9 cells. Such number of cells would be hard to obtain in a short time, their expansion in culture would take many days.

CONCLUSIONS

Although numerous doubts exists, the pre-clinical proofs of beneficial role of administered MSCs in ARDS are convincing. The therapy with these cells gives pleiotropic effects, achieved mainly by paracrine mechanisms. In the experimental models a several effects can be observed: limitation of pathological escalation of inflammatory reaction, enhancement of regeneration and support of antimicrobial defense mechanisms. Preliminary results of the clinical trials with MSCs in conditions like: graft versus host disease (48), diabetes (49), heart failure (50), infarction (51), stroke (52) are promising. Transplantation of MSCs in this conditions did not resulted in serious side-effects. It should be stressed that in all these cited trials a clinical benefit, at least in a groups of patients was observed. In conclusion, the unraveled features of MSCs suggest that the use of these cells in the therapy of ARDS could be almost ideal solution, however its introduction should be performed with cause. Undertaking the clinical trials with mesenchymal stem cells in ARDS is probably only a matter of time.

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