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ANALYSIS OF THE RESULTS OF MORPHOLOGICAL STUDIES AFTER THE INFLUENCE OF MESENCHIMAL STEM CELLS IN AN EXPERIMENTAL MODEL OF PULMONARY FIBROSIS

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Abstract. Aim: The aim of the study was to determine the optimal type of a nebulizer for viability of MSCs during nebulization, followed by a comparison of the effects of inhalation and intravenous delivery methods in a standard model of bleomycin pulmonary fibrosis in rabbits. Methods: At the first stage, the survival of MSCs was assessed ex vivo after 10 minutes of compressor, ultrasound and mesh nebulization. Subsequently we used a nebulizer, which showed the best result in the cells, viability. At the next stage after bronchoscopic installation of bleomycin, 5 rabbits received intravenous transplantation of 2×106 allogeneic BMMSCs, other 5 rabbits — 2×107 MSCs inhaled via a compressor nebulizer; the control healthy and bleomycin groups included 5 animals each. Results: The highest degree of viability of MSC was maintained after passing via the compressor nebulizer (72%), a significantly lower survival rate was observed in ultrasonic nebulization (20%) and no live cells were detected after mesh nebulization. Both groups treated with MSC had a significantly lower fibrosis index on the Ashcroft morphometric scale than the control group of bleomycin fibrosis. Collagen expression in the lung tissue was significantly higher in all the groups with bleomycin injury, but in animals which underwent MSC inhalation, it was significantly different (0.51 point) from the bleomycin group without treatment (2.1 points). Conclusions: The highest survival rate of MSCs is observed when using a compressor nebulizer, which apparently should be considered as the best way for delivering cells to the respiratory tract. Both inhalation and

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intravenous administration of MSCs cause similar effects of inhibiting the development of bleomycin-induced pulmonary fibrosis, which indicates the possibility of using both ways of cell delivery without loss of effectiveness.

Keywords: mesenchymal stem cells, pulmonary fibrosis, bleomycin, animal models, nebulizer

Introduction. Idiopathic pulmonary fibrosis is a chronic progressive disease of unknown etiology, characterized by the development of fibrous transformation of the pulmonary parenchyma, mainly in older persons [1, 11, 18, 19, 20, 21]. Despite the relatively low prevalence, the incidence of idiopathic pulmonary fibrosis is steadily increasing, on average by 5% per year, which is largely due to the general aging of the population [2, 9, 10, 12, 15]. Before the era of antifibrotic drugs, the median survival of patients with idiopathic pulmonary fibrosis from the time of diagnosis was 2.5–3.5 years [3, 5, 6, 8, 13, 14]. One of the promising approaches to the treatment of idiopathic pulmonary fibrosis and other fibrotic pulmonary diseases is the transplantation of various types of stem cells (usually mesenchymal), which, due to paracrine effects, can prevent or slow down the development of fibrosis in experimental models [4–6, 16, 17]. Thus, in one of the recent studies, mesenchymal cells of adipose tissue demonstrated an antifibrotic effect superior to that of the reference drug pirfenidone [7, 10]. It should be noted that the vast majority of preclinical studies were performed on a mouse model of bleomycin-induced pulmonary fibrosis, and the cell preparation was most often administered intravenously. Meanwhile, for humans, inhalation is one of the most common ways of delivering drugs to the lungs. However, with regard to cellular products, their delivery by nebulization through different types of inhalers has not been previously studied, the safety of cells after nebulization by heterogeneous physical methods has not been evaluated, and the effects of different routes of entry of biological material into the lungs have not been compared.

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In this work, the tasks were set to study the survival of mesenchymal stem cells (MSC) with various methods of nebulization, followed by the choice of the optimal inhaler and a comparative analysis of the effects of the cell preparation on the model of pulmonary fibrosis in rabbits with different routes of delivery to the lungs - inhalation and intravenous.

Methods

Research stages

The study consisted of 2 stages.

At the first stage, we assessed the survival rate of MSCs after their nebulization through different types of nebulizers. We used 3 nebulizers manufactured by OMRON Healthcare (Japan): compressor (jet) CompAir NEC24; ultrasonic UltraAir NE-U17 and mesh (mesh nebulizer) MicroAir U22. A suspension of 5 ml of 2 × 106 MSC in 0.9% sodium chloride (NaCl) was placed in the preparation chamber, adding as needed during the nebulization period. Spraying was carried out into a sealed plastic container for 10 min, from where 1 ml of aerosol condensate was taken for the subsequent assessment of cell survival. MSC viability was determined by staining cells with 0.4% trypan blue solution (Invitrogen Gibco, USA) followed by their counting in a Countess Automated Cell Counter (Invitrogen, USA) according to the manufacturer's instructions.

At the second stage, the effects of intravenous and inhalation (through the selected inhaler with the least damage to MSCs) methods of MSC administration were compared with respect to the development of local pulmonary fibrosis induced by endobronchial administration of bleomycin. The experiment was carried out on 20 mature chinchilla rabbits weighing 2.5-3.4 kg. Fibrobronchoscopy was performed on 15 rabbits under intravenous anesthesia (Nembutal, 50 mg / kg) in the supine position with an ultrathin fiberoptic bronchoscope with a diameter of 2.5 mm (model Karl Storz 6000V, Germany). Each animal was instilled into the posterior lobe of the right lung with a bleomycin

solution (Vero-Bleomycin, LENS-Pharm, Russia) at a dose of 2.5 mg / kg in 3 ml of 0.9% NaCl. The next day, 5 animals received intravenous injection into the ear vein of 2 million MSCs in 5 ml of 0.9% NaCl; another 5 rabbits underwent inhalation of 20 million MSCs in 5 ml of 0.9% NaCl, nebulized through a CompAir NE-C24 compressor nebulizer (OMRON Healthcare, Japan) using a neonatal mask. The remaining 10 animals were used as controls for bleomycin fibrosis (5) and healthy controls (5).

Results

Initially, the suspension prepared for spraying contained more than 95% of living cells. The best results were achieved using a conventional compressor nebulizer (72% of viable cells), while after nebulization with an ultrasound device, this figure was only 20%, and after passing through a mesh nebulizer, live MSCs were not detected.

At the second stage, when assessing the morphological changes in the lung tissue in the study groups, all animals with endobronchially administered bleomycin showed a pronounced thickening of the interalveolar septa, their infiltration and vessel walls with neutrophils and lymphocytes, as well as partial degradation of the acinus structure. In the groups that received intravenous or inhalation MSC treatment, these changes were present, but were significantly less pronounced than in the control group that underwent bleomycin administration without cell therapy. In addition to the described changes, massive fields of fibrosis with areas of complete obliteration of the alveoli and the vascular bed were revealed. In a semi-quantitative assessment of the degree of fibrosis in animals treated with MSCs, the severity of fibrosis was significantly less than in the control group of bleomycin fibrosis - 2.34 and 2.11 versus 4.15 points. A similar picture was observed when determining the degree of collagen expression: the maximum - in the group of bleomycin damage without treatment (2.1 versus 0.15 points in the healthy group), while in animals that received MSCs, this indicator was

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significantly lower - 0, 79 points for intravenous therapy and 0.51 points for inhalation delivery (Fig. 4). The cytokine profile of the bronchoalveolar lavage fluid turned out to be less informative due to the fact that TNFα and IL6 (pg / ml) were not detected in healthy rabbits, and although the TGFβ1 parameters were quantitatively higher in the groups receiving bleomycin, they did not differ significantly among themselves.

The results of cytological analysis of bronchoalveolar lavage fluid are presented. The total cytosis in the bleomycin group without treatment was significantly higher than in the healthy control (567 versus 365 cells / μL); however, this indicator in the MSC treatment groups, although it tended to decrease, did not reach statistically significant values. Similar changes were observed in cellular fractions.

Conclusion

Our study, on the one hand, proved the feasibility of using a compressor inhaler to deliver a maximum of viable cells to the respiratory tract, on the other hand, demonstrated comparable positive morphological, immunohistochemical and cytological effects of intravenous and inhalation routes of administration of allogeneic bone marrow MSCs in a model of bleomycin-induced pulmonary fibrosis in rabbits. The positive results of cell therapy in larger animals than in previous studies, as well as the technology for delivering cells to the respiratory tract, which is acceptable for humans, allow us to hope for the possibility of a transition from preclinical to clinical studies in this direction.

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