Off-label mesenchymal stromal cell treatment in two infants with severe bronchopulmonary dysplasia: clinical course and biomarkers profile

MARIA ÁLVAREZ-FUENTE1, LUIS ARRUZA2, PALOMA LOPEZ-ORTEGO3, LAURA MORENO4, MANUEL RAMÍREZ-ORELLANA5, CARLOS LABRANDERO6, ÁFRICA GONZÁLEZ5, GUSTAVO MELEN5 & MARIA JESÚS DEL CERRO1

1Pediatric Cardiology Department, Ramón y Cajal University Hospital, Madrid, Spain, 2Neonatology Department, Hospital Clínico San Carlos, Madrid, Spain, 3Neonatology Department, La Paz University Hospital, Madrid, Spain, 4Department of Pharmacology, School of Medicine, University Complutense of Madrid, Instituto de Investigación Sanitaria Gregorio Marañón (ISGGM), Ciber Enfermedades Respiratorias (CIBERES), Madrid, Spain, 5Cell & Gene Therapies Laboratory, Niño Jesús University Hospital, Madrid, Spain, and 6Pediatric Cardiology Department, La Paz University Hospital, Madrid, Spain

Abstract

Background: Bronchopulmonary dysplasia (BPD) is the most prevalent sequela of premature birth, for which therapeutic options are currently limited. Mesenchymal stromal cells (MSCs) are a potential therapy for prevention or reversal of BPD. Series of cases: We report on two infants with severe BPD in whom off-label treatment with repeated intravenous doses of allogeneic bone marrow–derived MSCs were administered. We analyzed the temporal profile of serum and tracheal cytokines and growth factors as well as safety, tolerability and clinical response. The administration of repeated intravenous doses of MSCs in two human babies with severe and advanced BPD was feasible and safe and was associated with a decrease of pro-inflammatory molecules and lung injury biomarkers. Both patients were at very advanced stages of BPD with very severe lung fibrosis and did not survive the disease. Conclusions: MSCs are a promising therapy for BPD, but they should be administered in early stages of the disease.

Key Words: bronchopulmonary dysplasia, chronic lung disease of the newborn, mesenchymal stromal cells, molecular biomarkers, preterm newborns, pulmonary vascular disease, regenerative medicine, very low–birth weight infants

Introduction

Despite the increase in the survival of extremely premature babies in the past decades, morbidity and mortality still remain high in these patients [1]. Bronchopulmonary dysplasia (BPD) is the most prevalent sequela of premature birth [2–5], and the most common cause of chronic lung disease in infancy. Between 18% and 37% of the patients with BPD will develop pulmonary hypertension (PH) [6–10], which worsens the prognosis [11–13].

Although significant advances in the understanding of BPD have been made, therapeutic options are currently limited. There has been increasing interest in the potential role of mesenchymal stromal cells (MSCs) in the prevention or reversal of BPD. The current assumption is that MSCs are active cells that can sense signals in the surrounding tissues and modulate their paracrine actions in response, secret ing mediators able to reduce inflammation and repair injured tissues [14–16]. Different experimental animal models have shown the ability of MSCs to improve lung and cardiac function (reducing fibrosis, promoting the normal development of alveoli and pulmonary vessels and ameliorating PH and its consequences on cardiac function) in animal models of BPD and lung injury [17–21]. To date, only one human study has evaluated the effect of intratracheal administration of umbilical cord–derived MSCs in extremely premature babies at high risk of developing severe BPD [22]. This phase 1 trial reported short-term safety and tolerability, and suggested a beneficial effect of this therapy compared with historical controls, with a decrease in BPD severity and a
reduction in pro-inflammatory cytokines in tracheal aspirates. The extension study showed safety and no adverse respiratory, growth or neurodevelopmental effects at 2 years of follow-up [23]. Two clinical trials focusing on MSCs and BPD are currently ongoing (NCT01828957 and NCT02381366).

Herein, we report on two infants with severe BPD in whom off-label treatment with repeated doses of allogeneic bone marrow–derived MSCs were performed. We analyzed the temporal profile of serum and tracheal aspirate cytokines and growth factors as well as safety, tolerability and clinical response.

Description of cases

Patient 1

This patient was a preterm female born at 24 + 3 weeks gestational age (GA) due to placental abruption with umbilical cord prolapse after premature rupture of membranes at 20 weeks GA. Complete prenatal steroid treatment (two doses 24 h pre-labor) was administered. Birth weight was 695 g. Chest X-ray showed signs of respiratory distress syndrome (RDS; Figure 1A). Surfactant (200 mg/kg) was given 1 h after birth.

The patient developed severe BPD associated with severe PH, treated with oral sildenafil and bosentan. A computed tomography (CT) scan performed at 4 months of age ruled out airway or pulmonary vein stenosis, aortopulmonary collaterals and patent ductus arteriosus (PDA). The scan identified diffuse and severe parenchymal disease with multifocal hyperlucent areas, linear opacities and subpleural opacities, scarring bullae and mild dilatation of the pulmonary trunk and pulmonary branches (Figure 1A). At 5 months of age, due to the unfavorable
evolution and dismal prognosis, the parents were offered off-label treatment with allogeneic, human bone marrow–derived MSCs (male donor). After detailed information on the experimental nature of the treatment, the parents consented. A specific experimental protocol of increasing weekly doses of intravenous MSCs was designed for this patient. Table 1 shows details of preparation and administration of MSCs.

Considering the lack of experience with this treatment and how this treatment is administered in other pediatric indications, such as pediatric oncology, we considered necessary the premedication with steroids to avoid any potential allergic reaction. Therefore, this patient received prednisolone (0.5 mg/kg) 30 min before every cell infusion. No other anti-inflammatory treatments or inhaled nitric oxide (iNO) were administered simultaneously. The respiratory and cardiac evolution, before and during the 5 weeks of treatment, is shown in Figures 1B and 1C.

No acute adverse reactions related to the MSC infusion occurred. The patient did not present evident respiratory improvement. Only mild improvement of PH echocardiographic parameters was observed after the first three doses (Figure 1B).

Gene expression profiles in peripheral blood cells before and after each MSC dose are shown in Figure 3. Due to the small volume of the blood samples, proteins were not measured in plasma, but instead messenger RNA (mRNA) levels were obtained from blood cells. The patient showed a reduction in mRNA levels of the inflammatory cytokines interleukin (IL)-1β and High Mobility Group Box 1 Protein (HMGB1), in the levels of the endogenous antagonist of Vascular Endothelial Growth Factor (sVEGFR1) and in the innate immune receptor NLR Family Pyrin Domain Containing Protein 3 (NLRP3), whereas the expression of IL-6 mRNA was transiently increased after the first dose. The patient showed positive mRNA surfactant protein D (SP-D) expression in peripheral blood cells prior to treatment with MSCs; by the end of the MSC treatment course this protein was undetectable. In contrast, the levels of acid sphingomyelinase (SMPD1), an enzyme generating ceramide, involved in pulmonary edema formation, were increased in peripheral blood cells.

The patient died 6 weeks after the beginning of MSC therapy, due to a pneumothorax and to the parent’s decision to redirect patient care. A necropsy was performed and lung histology showed cystic areas of emphysema, bronchiolar smooth muscle hypertrophy and simplified alveolar structure, with a decrease in the number of alveoli, interstitial cell proliferation and fibrosis (Figure 1D). Genomic DNA was isolated from two different frozen lung tissue sections with an Ambion modified protocol (Ambion Inc). Devyser Compact v3 QF-PCR kit (Devysen AB) was used to polymerase chain reaction (PCR) amplify autosomal and gender chromosome markers and to determine the gender dosage in the cells of lung tissue. Amplification products sizes were determined in a ABI3130xl Genetic Analyzer (Applied Biosystems Inc.). Absence of SRY (Yp11.31) and AMELY (Yp11.2), a normal diallelic pattern for markers DXS1187 (Xq26.2), XHPRT (Xq26.2-q26.3) and DXS2390 (Xq27.1-q27.2), and a 1:1 dosage ratio both between chromosomes 7q34 and Xq13, and 3p24.2 and Xq21.1, demonstrated a normal female pattern and no male-specific markers on the DNA obtained from both tissue samples, suggesting the absence of engraftment of the administered allogeneic MSCs from the male donor.

### Patient 2

This patient was a preterm female born at 24 + 2 weeks of GA by spontaneous delivery due to bulging membranes. A single dose of antenatal steroids was administered. Birth weight was 700 g. Placental pathology showed signs of choioamnionitis.

The patient developed severe BPD associated with severe PH. A CT scan performed at 80 days of life showed lung fibrosis with areas of

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
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<tbody>
<tr>
<td><strong>Source of MSCs</strong></td>
<td>Healthy bone marrow male donor</td>
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<tr>
<td><strong>MSC preparation</strong></td>
<td>MSCs were prepared and suspended in sterile saline infusion</td>
</tr>
<tr>
<td><strong>Route and mode of administration</strong></td>
<td>Slow i.v. infusion (1 h) through a central jugular vein catheter with platelet filter</td>
</tr>
<tr>
<td><strong>Premedication</strong></td>
<td>i.v. ranitidine + i.v. prednisolone 30 min before infusion</td>
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<tr>
<td><strong>Dose</strong></td>
<td>Increasing weekly dose: 1.1 million cells/kg up to 13.9 million cells/kg</td>
</tr>
<tr>
<td><strong>Monitoring during infusion</strong></td>
<td>SpO2, blood pressure, echocardiography</td>
</tr>
<tr>
<td><strong>Adverse effects</strong></td>
<td>None</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>5 million cells/kg per week for 3 consecutive weeks</td>
</tr>
<tr>
<td><strong>Monitoring during infusion</strong></td>
<td>None</td>
</tr>
</tbody>
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i.v., intravenous; SpO2, oxygen saturation.
overinflation combined with atelectasis ruling out pulmonary to systemic collaterals, airway or pulmonary vein stenosis (Figure 2A). At 85 days of life, due to persistent hypoxemia despite aggressive ventilatory support, off-label treatment with intravenous administration of repeated doses of allogeneic bone marrow–derived MSCs was offered, approved by the parents and initiated. Considering our first experience and the clinical trial published by Chang et al. [22], which was published between both patients, we established a different treatment protocol in this patient (Table 1). In this clinical trial the patients received two different doses: 10 and 20 million cells/kg [22]. They did not prove to have better results at a higher dose [22]. In our experience (case 1) we also did not achieve better results at higher MSC doses. Therefore, we opted for a total dose of 15 million cells/kg divided in three doses. In addition, considering the low immunogenicity of MSCs and the lack of allergic reactions in the clinical trial [22,24], in which no premedication with steroids was administered, we decided not to administer steroids in patient 2.

The evolution of the respiratory parameters before and during the MSC treatment is shown in Figure 2B. No acute adverse reactions related to the MSC administration were observed. A reduction in inflammatory biomarkers (IL-1β, IL-6, HMGB1 and NLRP3) in peripheral blood cells was observed by the end of the treatment with MSCs (Figure 3). In tracheal aspirates, IL-6 protein levels were transiently reduced, whereas IL-1β levels were increased by the end of the study period. In this patient, after receiving three doses of MSCs, mRNA SP-D in peripheral blood cells was undetectable. Similarly, the circulating levels of sVEGFR1 and SMPD1 decreased after the MSC infusions.
At 4 months of age, 3 weeks after the last MSC infusion, the baby presented with further respiratory deterioration with hypoxemia despite 100% oxygen inhaled fraction (FiO2) and 20 ppm iNO. The parents’ decision was to redirect patient care and the baby died.

Discussion

This is the first report on the use of intravenously delivered MSC administration in two preterm infants with severe BPD. It is also the first report on repeated MSC doses administration in preterm babies. Our experience provides information on feasibility and safety of repeated doses of MSCs using the intravenous route.

Route of administration

The optimal route of MSC administration remains unclear. We have explored the intravenous route, considering that the infused cells will easily reach the lung. In animal biodistribution studies, after intravenous administration, MSCs were detected in the lung during the first 24 h and no residual cells were found in any organs 7 days after the infusion [25–27]. These studies also show that the majority of the cells administered intravenously will be filtered by the lung and that very few reach other organs, mostly the liver and the spleen for less than 7 days [27–29].

Our study group studied the biodistribution, biosafety, and tumorigenicity of human umbilical cord–derived MSCs (UC-MSCs) in immunodeficient mice. Five million cells per kilogram of luciferase-expressing UC-MSCs were intravenously inoculated in 10- to 12-week-old NOD.Cg-PrkdcsidIl2rgtm1Wjil/SzJ mice. Mice underwent bioluminescent study every day for the first 4 days and then weekly for a 3-month period. We observed an accumulation of luminescent signal in mice lungs during the first hours post-infusion. Although luminescent signal was acquired during the 3 months, its activity disappeared over the first week. No accumulation of UC-MSCs was observed in other organs. With our experience we can conclude that after intravenous
administration, most MSCs were filtered by the lung capillaries, and stayed there alive for 4 to 7 days, therefore, intravenous administration is appropriate when the lung is the target organ. Also, considering that UC-MSCs will disappear after 7 days, repeated weekly doses should be considered to maintain their therapeutic effect. A more detailed description of our research on biodistribution has been submitted and is currently being evaluated for publication.

On the other hand, the clinical trial performed by Chang et al. suggests the safety of allogeneic cord blood–derived MSCs via the intratracheal route in short- [22] and medium-term follow-up (2 years) in 9 preterm infants. This research group also published the comparison of intratracheal versus intravenous route of cord blood–derived MSCs in hypoxic neonatal rodents [29]. They found that the intratracheal route was more efficient in terms of number of labelled membrane particles detected in the animal lungs.

Nevertheless, both routes of administration showed the beneficial effects of MSCs (improvement in alveolarization, inflammatory response and apoptosis) and had similar survival rates. The intratracheal route is appealing in terms of safety (potentially avoiding cell dissemination to other organs) and feasibility in intubated babies, whereas the intravenous route makes feasible the treatment of infants on non-invasive ventilation modalities.

Determining the optimal administration route, dose and timing of MSC therapy still remains to be explored in relevant pre-clinical models and/or in well-designed clinical trials.

**Timing of administration**

Although MSC administration was well tolerated, it did not change the fatal disease course of our patients. A recently published systematic review and meta-analysis showed that, in animal models of BPD, MSC treatment administered after hypoxia exposure favored lung alveolarization and lung angiogenesis and diminished lung inflammation, fibrosis and PH [30]. Our patients were at very advanced and severe BPD stages; therefore, we speculate that when lung damage is advanced, and fibrosis is already well established, MSC therapy is not effective, although it diminishes systemic inflammation. Considering the role of MSCs in lung development, we hypothesize that the administration of MSCs in patients with BPD should be performed at earlier stages of the disease or even prophylactically, in patients with high risk of BPD to modulate the repair response and prevent irreversible tissue remodeling.

In our patients MSC administration was performed very late at an advanced stage of the disease, in which established lung injury with large areas of fibrosis may no longer be reversible. Nevertheless, in case 2, in which MSC administration was performed at 83 days postnatal age, we observed a decrease in the oxygen need and respiratory support in the weeks during MSC administration. This effect vanished when MSC administration was interrupted (Figure 2B). Given the complex pathophysiology of human BPD and the transitory effect of MSC, repeated MSC administration may be worth considering once more data on safety of MSC administration are available.

**Toxicity**

Regarding safety, we observed no acute adverse reactions during intravenous administration. In patient 1, when observing Figure 1C, we can see that after four of the six doses there was a small increase in the respiratory severity score, which could be interpreted as an adverse effect of the MSCs. Considering that we did not observe this after every administration nor in patient 2, and that patient 1 was at a very poor respiratory condition, we consider that other factors could have been responsible for this increase.

Another important safety issue is the potential tumorigenic effect of MSCs. The genomic amplification techniques performed in the lung tissue of patient 1 suggest no engraftment of the infused cells, further supporting possible long-term safety given the absence of cells from which abnormal proliferation could originate. A clinical trial with long-term follow-up would be required to be able to confirm the long-term safety of this therapy.

**Inflammatory biomarkers**

We observed in both patients a systemic decrease in blood levels of pro-inflammatory biomarkers (Figure 3). Although the reduction in IL-1β and HMGB1 levels was more pronounced in the patient receiving concomitant corticosteroids, this has to be taken into account when making conclusions. Other inflammatory biomarkers, such as IL-6 and NLRP3, were similarly reduced by MSCs in both patients. Similar results were observed by Chang et al., who found a significant decrease of IL-6, IL-8, IL-10, IL-1β, tumor necrosis factor (TNF)α and transforming growth factor (TGF)β 7 days after MSC administration [23]. We also observed in our patients that the expression levels of SP-D mRNA in peripheral blood cells, which reflect the presence of lung cells in the systemic circulation in patients with lung cancer or lung injury [31,32], were nearly abolished after three doses of MSC administration. In contrast, the expression levels of SMPD1, an enzyme generating
ceramid and involved in pulmonary edema formation and acute PH [33–35], were found to be increased in the patient receiving both MSCs and corticoids but it was reduced in the patient receiving only MSCs (case 2) coinciding with a mild improvement in oxygenation and ventilatory support needs in this patient. Ceramides have also been recently found to be biomarkers predictive of BPD [36]. Although the impact of these findings remains unclear, it should be noted that these results are in line with those reported from the European multicenter trial of nitric oxide (NO) inhalation for preterm infants to prevent BPD [35], where a transient reduction in inflammatory markers by iNO treatment was accompanied by a delayed increase in SMPD1 tracheal aspirate levels. The reduction of systemic inflammation is very promising not only in the prevention of premature lung disease, but also in the deleterious effect of inflammation in other organs.

Limitations

The main limitation of our experience is that both patients had very advanced BPD with established fibrosis. Therefore, efficacy of MSC therapy cannot be evaluated. In addition, treatment in only two patients is not enough to test efficacy. On the other hand, these experiences have shown the lack of toxicity with the intravenous administration of MSCs in two very sick patients with advanced severe BPD.

Another limitation is the administration of steroids on patient 1 that could have biased the results of the anti-inflammatory biomarkers. For future clinical trials this should be taken into account.

In conclusion, the administration of repeated intravenous doses of MSCs in two human babies with severe and advanced BPD was feasible, no toxicity was observed and it was associated with a decrease in the levels of pro-inflammatory molecules and biomarkers of lung injury. MSCs are a promising therapy for BPD, but they should be administered in the early stages of the disease. The use of molecular biomarkers in response to MSCs in the preterm lung could be useful to elucidate the mechanisms of action of these cells.

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References


