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Case Report / Olgu Sunumu

The clinical and radiological course of bronchopulmonary dysplasia in twins treated with mesenchymal stem cells and followed up using lung ultrasonography

Bronkopulmoner displazi tanılı ikizde mezenkimal kök hücre tedavisi ve akciğer ultrasonografisi ile tedavi izlemi

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The known about this topic

Stem cells have a high potential for growth due to the high telomerase enzyme activity they possess. The function of differentiation of stem cells is provided by expression of transcription factors depending on the activation of signal pathways originating from the microenvironment in which they are found.

Contribution of the study

The contribution of this study to the literature is to show the efficiency of stem cells in the treatment of BPD in preterm patients with very low birth weight. In addition to findings of clinical improvement, lung radiography findings and lung ultrasonography findings will provide support to studies in this area. Lung ultrasonography findings following stem cell treatment were demonstrated and evaluated in preterm babies.

Abstract

Bronchopulmonary dysplasia is a chronic lung disease that develops in low-birth-weight infants as a result of mechanical ventilation and oxygen toxicity in the early neonatal period. In these patients, mechanical ventilation and oxygen support are needed for a long time. We already use antenatal steroid, ventilation techniques with minimal baro/volutrauma, postnatal steroid, and vitamin A to prevent the development of bronchopulmonary dysplasia. Mesenchymal stem cell treatment is another way to reduce or stop the pathophysiologic pathways in the development of bronchopulmonary dysplasia. Herein, we present mesenchymal stem cell treatment and its outcomes in twins who were born with a gestational age of 26 weeks and diagnosed as having bronchopulmonary dysplasia (the female twin was born with a birth weight of 750 g and the male twin was born with a birth weight of 930 g). These patients were followed up with clinical findings, chest radiography, and lung ultrasonography.

Keywords: Bronchopulmonary dysplasia, lung ultrasonography, mesenchymal stem cells

Öz

Bronkopulmoner displazi düşük doğum ağırlıklı bebeklerde erken yenidoğan döneminde mekanik ventilasyonun etkisi ve oksijen toksititesi gibi nedenlerle ortaya çıkan kronik bir akciğer hastalığıdır. Bu hastalarda ventilasyon desteği ve oksijen ihtiyacı uzun süre devam etmektedir. Bronkopulmoner displazi gelişimini engellemek amaçlı antenatal steroid kullanımı, baro/volutravmayı azaltacak ventilasyon teknikleri, postnatal steroid ve A vitamini kullanımı gibi stratejiler vardır. Son yıllarda, bronkopulmoner displazi gelişimindeki patofizyolojik mekanizmaların durdurulması ve/veya azaltılması amaçlı tercih edilebilecek yöntemlerden birtanesi de mezenkimal kök hücre kullanımıdır. Bu yazıda, gebelik haftası 26 olan 750 g kız ve 930 g erkek doğum ağırlığına sahip ikizlere, bronkopulmoner displazi tanısıyla verilen mezenkimal kök hücre tedavisi ve sonuçları sunulmuştur. Bu hastalar klinik bulgular, akciğer grafileri yanı sıra tekrarlayan akciğer ultrasonografisi ile izlenmiştir.

Anahtar sözcükler: Akciğer ultrasonografisi, bronkopulmoner displazi, mezenkimal kök hücre

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Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that develops in low-birth-weight infants as a result of causes such as mechanical ventilation and oxygen toxicity in the early neonatal period. Classic-type BPD was first described in 1967 by Northway et al. (1). In the

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definition of classic BPD, it was observed that BPD developed as a result of ventilation using high pressure and oxygen because of respiratory distress syndrome (RDS) in babies born at the 30–37th gestational week. The concept of 'new BPD' emerged in preterm babies with very young gestational age (24–28 gestational weeks) with antenatal steroid treatment, surfactant applications, and the development of milder ventilation techniques. In these cases, it was observed that babies initially did not have RSD or had mild RDS, but disruption in pulmonary functions and an increase in oxygen need occurred after a short while. Sepsis or patent ductus arteriosus (PDA) may accompany this picture (2). In addition to various treatment protocols used in the treatment of BPD, mesenchymal stem cell (MSC) treatment has been used in recent years to decrease pulmonary inflammation and to reduce pulmonary hypertension (3). In this article, we present MSC treatment that was used in two patients with a diagnosis of BPD, the outcomes of this treatment, and the follow-up of the patients with lung ultrasonography (USG).

Case

A female baby with a birth weight of 750 g and a male baby with a birth weight of 930 g were born at the gestational age of 26 weeks from a 29-year-old mother. Pregnancy was complicated by the preterm rupture of the membranes. The twins were resuscitated after birth, and they were intubated and surfactant therapy was administered. The male patient was extubated on the fourth day and was started to be followed up with nasal continuous positive airway pressure (CPAP). On the postnatal 28th day, the male patient was separated from CPAP and was started to be followed up with free oxygen. However, mechanical ventilation was continued in the female baby. The patients' lung radiographic and clinical findings were compatible with severe BPD. It was decided to give MSC treatment on the postnatal 32nd day to both patients. Mesenchymal stem cells (2x10^6/kg) were given by the intravenous route and 1x10^7/kg MSCs were given by the intrathecal route. The patients were monitored using lung USG and the images were recorded.

Atelectasis, consolidation, pleural line abnormalities, alveolar interstitial syndrome (AIS), B lines, and air bronchograms were seen on USG examinations of the lung in both patients before treatment. No significant change was found in lung USG repeated twelve hours after stem cell treatment (Fig. 1a, b). Ultrasound examination of the lung performed in the female patient three days after treatment and revealed pulmonary consolidation, partial resolution in divided areas, atelectatic regions, and also, it was observed that pleural line abnormalities persisted. In the male patient, lung USG revealed that B lines and AIS disappeared, but pleural line irregularity persisted.

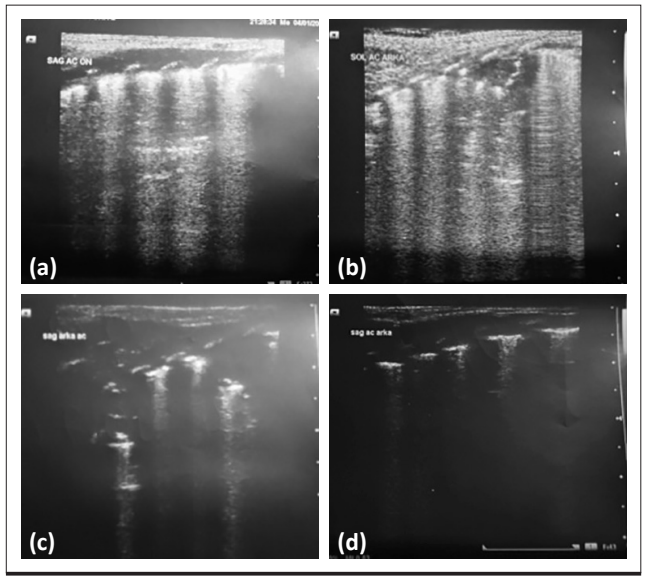

Figure 1. (a) Lung USG of the male patient before stem cell treatment (lung ultrasonography reveals prominent B lines, pleural line is not regular, there are consolidation areas involving air bronchogram, A lines, which are observed in normal lung tissue, are absent). (b) Lung USG of the female patient before stem cell treatment (lung ultrasonography shows irregularities in lung parenchyma and pleural line. Widespread B lines and consolidation areas involving air bronchogram are present. A lines, which are observed in normal lung tissue, are absent). (c) Lung USG of the female patient 15 days after stem cell treatment (irregularities in lung parenchyma and pleural line persist on lung ultrasonography. There is a reduction in the number of B lines and the appearance of A lines is increased. However, the clinical findings of this patient had a more severe course in accordance with the ultrasonographic imaging compared with her brother). (d) Lung USG of the male patient 15 days after MSCs treatment (lung ultrasonography revealed that the number of B lines was reduced, the 'lung sliding' sign developed, A lines appeared, comet tail was observed in the area of imaging, and consolidation areas were deleted)

Lung USG of the female patient performed nine days after the treatment showed that pleural line irregularity reduced, A lines became prominent, and AIS areas gave place to B lines, and the patient's need for oxygen decreased. A USG examination of the male patient revealed that A lines started to become prominent and AIS areas were replaced by B lines. Simultaneous echocardiography was planned for the patients. Hemodynamically significant PDA was found on echocardiography in the female patient, and the male patient had PDA that could be controlled by medical treatment. On the fifteenth day of treatment, a final follow-up USG examination was performed in the patients.

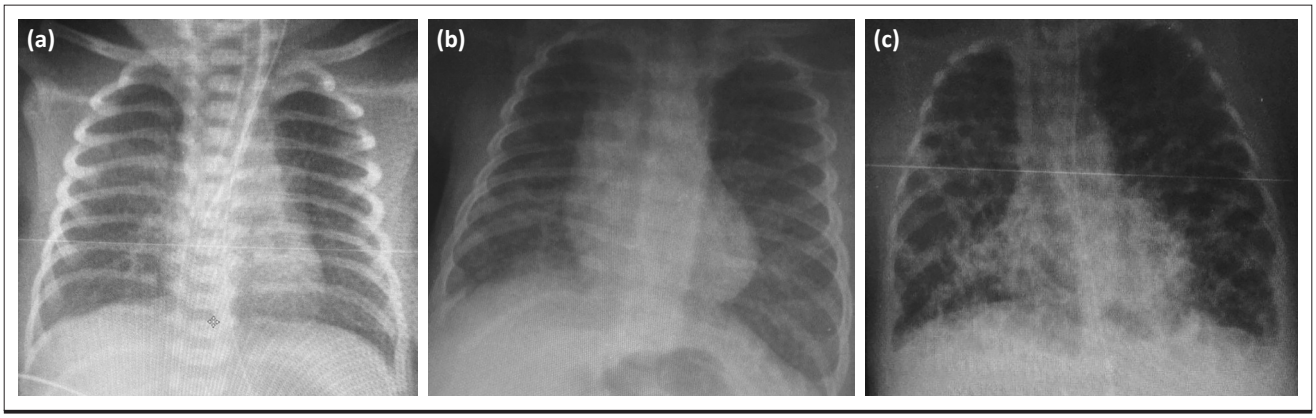

Figure 2. (a) Chest radiography of the male patient before stem cell treatment. (b) Follow-up chest radiography of the male patient after stem cell treatment before discharge. (c) Chest radiography of the female patient before stem cell treatment

It was observed that AIS areas were completely replaced by B lines, A lines became more prominent, and air bronchograms were present in pulmonary consolidation areas in the female patient (Fig. 1c). However, the patient died of sepsis on the 18th day of treatment. The male patient's lung ultrasonographic findings were almost normal (Fig. 1d), his oxygen need was reduced and he was receiving free oxygen only during feeding. Subsequently, the patient was discharged after discontinuing oxygen support completely. Written informed consent was obtained from the parents to publish the findings and results of the patients. Mesenchymal stem cells to be used within the context of MSC treatment were obtained from Acıbadem Labmed Health Services Cell Laboratory.

The method of obtaining MSCs

As the cells cultured by mechanical and enzymatic procedures from the cord blood are observed to have covered 70% of the flask base, they are included in the first passage procedure; 3000–5000 cells/cm^2 are planted, cultured with the relevant medium, and stored in an incubator. When they are observed to have covered 70% of the flask base five days later, they are included in the termination procedure. The MSCs obtained at the end of the procedure are diluted with isotonic solution and quality control samples are given (Cell number, Viability, Flow Cytometry, LAL; Mycoplasma, Microbiological quality control).

Discussion

Bronchopulmonary dysplasia is an important respiratory tract pathology, especially in very-low-birth-weight newborns. The patients presented in this article had risk factors such as small gestational age and very low birth weight. The patients were resuscitated after birth and they needed mechanical ventilation in the early post-natal period. Although prematurity, trauma and oxygen toxicity are the most important factors in the development of BPD, antenatal and/or postnatal inflammation, infections, PDA and nutritional problems are also involved in the etiopathogenesis (2). Betamethasone was administered to the patients' mother twice with an interval of 12 hours before delivery. Although it has been noted that the use of antenatal steroid reduces the development of RDS, its effects on the development of BPD are not clear. Antenatal steroid prevents RDS by providing earlier than normal lung maturation, but BPD develops because a lung with abnormal structure emerges. Lung radiographs give information as well as the patient's clinical status in the diagnosis of BPD. In direct radiography, the most common findings include fine/coarse densities extending to the periphery of the lung, over-aeration and non-homogeneous appearance of the lungs, cystic formations, and fibrotic changes of different degrees (2, 4).

These patients had similar lung radiography findings (Fig. 2), and as a new approach in the literature, they were followed up in the neonatal intensive care unit using bedside lung USG, used in the diagnosis of many different diseases in newborns. Lung USG is advantageous because it is an easily applicable bedside method, does not involve radiation and is inexpensive. Lung USG may reveal consolidation areas, pleural line irregularities, air bronchograms, AIS, B lines, and cystic structures in patients with BPD (4). We found the same findings on lung USG in patients before treatment. On the 15th day of treatment, follow-up USG examination revealed that the findings regressed, AIS areas were replaced by B lines (which are a milder form of AIS), A lines became more prominent, and air bronchograms in lung consolidation areas persisted.

One of the newest applications performed to prevent the development of BPD is mesenchymal stem cell treatment. Many studies have shown that stem cells and mediators released from these cells can provide hemostasis

by downgrading lung damage in BPD, and thus reduce lung injury, fibrosis, and pulmonary hypertension (5). Bronchopulmonary dysplasia is also characterized by an abnormal distribution of pulmonary vasculature and a decrease in pulmonary small arteries, which causes pulmonary hypertension. Pulmonary vasculature decreases, and wall thickness increases. As a result, increased pressure leads to right ventricular hypertrophy (6). In a series including nine cases presented by Chang et al. (7), MSCs prepared from umbilical cord blood were administered by the intrathecal route to prevent the development of BPD, and it was observed that the picture of BPD was milder and a significant reduction occurred in the duration of intubation, nasal ventilation, and the use of postnatal steroids. Stem cells protect alveolar epithelial cells and microvascular endothelial cells from oxidative stress by paracrine effects and can stimulate bronchoalveolar stem cells which support the lung repair. Thus, pathologies related to hyperoxia such as fibrosis, inflammation, alveolar damage and pulmonary edema may be reduced with MSCs treatment and pulmonary functions and survival rates are increased (8). Mediators released from MSCs such as keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and epidermal growth factor (EGF), involves in the lung repair through paracrine action. Keratinocyte growth factor increases lung maturation additionally. It has been observed that prenatal dexamethasone use increases KGF mRNA expression in fetal lung fibroblasts, and thus maturation of distal lung epithelium increases. Vascular endothelial growth factor acts by way of two tyrosine kinase receptors found on endothelial cells including vascular endothelial growth factor–receptor 1 (VEGF-R1) and VEGF-R2. VEGF-R1 regulates endothelial intercellular signal pathways, which are important for vascular development, and VEGF-R2 provides endothelial cell mitogenesis and lung maturation (9). Angiopoietin-1 reduces apoptosis in human vein endothelial cells. In this way, it reduces inflammation and lung injury by preserving barrier functions that control protein passage in endothelial and epithelial cells. Epidermal growth factor is another important epithelial growth factor that accelerates lung development. EGF receptor expression is reduced in fetal lungs with pulmonary hypoplasia (8). Again, Leeman et al. (10) added MSCs to lung progenitor cell cultures and showed that alveolar differentiation increased with cytokines released from stem cells. In this case, MSCs may accelerate alveolar regeneration in the treatment of lung injury. Mesenchymal stem cells, which have been used in the treatment of many diseases, gain importance in newborns with BPD in recent years. In this case presentation, we aimed to emphasize that MSC treatment can be used in the treatment of BPD and lung findings can be followed up with USG in newborns. However, prospective, multi-center, controlled studies including the efficiency and long-term outcomes of MSC treatment should be conducted in the future.

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