Respiratory dystrophy

Neurodegenerative

Amyotrophic

Fetal stem cells are effective in the treatment of Grade I and II respiratory failure in amyotrophic lateral sclerosis and muscular dystrophy

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ABSTRACT

Objectives: To study the effect of fetal stem cell (FSC) therapy on Grade I and II respiratory failure in patients with amyotrophic lateral sclerosis (ALS) and muscular dystrophy (MD).

Methods: A comparative study was conducted on 41 patients with Grade I or II respiratory failure (RF) resulting from ALS or MD. The patients were divided into 4 groups according to the underlying disease and the degree of RF. Patients underwent combined treatment, including the experimental application of FSC therapy, and were examined before FSC treatment, and 6 months and 12 months after treatment.

Results: FSC treatment improved both subjective and objective breathing parameters as early as 6 months post-treatment. A significant increase in the forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) was reported by all patients with grade I RF linked to ALS and MD compared to baseline. Patient respiratory improvement was maintained over the next 6 months. Grade II RF patients with MD reported a significant improvement in FVC 12 months after treatment.

Conclusions: Evidence for respiratory improvement was observed as early as 6 months in all patients after combined treatment including FSC therapy, and this was maintained for a further 6 months after therapy. In MD patients with Grade II RF, treatment resulted in a significant FVC and FEV1 increase within 6 months and downgrading to Grade I RF within a year after FSC treatment.


1 Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease resulting in respiratory failure (RF). It is caused by the loss of upper and lower motor neurons that innervate the respiratory muscles and coordinate contractile activity of the pharynx, larynx and tongue muscles, which are...
responsibility for the coughing reflex[1–5]. ALS is an incapacitating disease among individuals of working age and rapidly results in mortality. Without specialized medical care and devices, the average life expectancy of ALS patients does not exceed 2.5–3 years if there is a bulbar disease onset, or 3.5 years if there is a spinal onset. Only 7% of ALS patients live longer than 60 months[6].

RF is defined as inadequate gas exchange by the respiratory system, or its maintenance only through excessive work, often resulting in apnea. RF is often found in patients with ALS and muscular dystrophy (MD), and is the main cause of death in ALS patients[7].

MD represents a group of chronic hereditary diseases that can affect skeletal muscles leading to progressive weakness and muscular degeneration. This degeneration can spread to the upper body, gradually involving all of the main muscle groups, including those required for respiration. This disease characteristically causes damage to the voluntary and smooth muscles and myocardium, ultimately leading to respiratory excursion, and a decompensation state. In MD, RF is caused by muscle tissue damage resulting from a mutation in the dystrophin gene[8–9]. Muscle fibers become necrotic and are substituted by fatty and connective tissue, which results in impaired respiration[8]. RF lowers the overall life expectancy in MD patients irrespective of its type, and its progression results in cardiopulmonary decompensation in most cases[9–10].

Respiratory disturbances are fatal in both ALS and MD. Although respiratory exercises and oxygen therapy are recommended for these patients, an effective method to prevent ALS and MD progression and the inevitable development of RF remains to be found. Suggested alternative therapeutic methods include fetal stem cell (FSC) transplant, and several studies have explored this possibility.

Specialists at the A. P. Romodanov Institute for Neurosurgery (Ukrainian National Academy of Medical Science) developed the stem cell technology used for the treatment of Duchenne MD. The biological formulation consisted of stem cells and myoblasts that were injected directly into the muscle tissue, preventing myocyte destruction. This in turn led to disease remission[11]. It is assumed that stem cells contain specific muscle markers and are capable of dystrophin expression[12–14]. Other researchers have used both intravenous and intramuscular modes of FSC administration[15]. Hematopoietic and mesenchymal FSCs can reach “niches” throughout the circulation and then become satellite cells during the course of development. These in turn usually transform into muscle fibers. The extent to which they migrate depends on the degree of muscle impairment[16].

Fetal myoblasts have several advantages over embryonic ones[17–18]. They have a triangular shape and can divide several times in a non-differentiated state, and then differentiate into multinuclear myocytes in response to growth factors[17–18]. Fetal neuronal stem cells have the potential to differentiate into neural cells inside the impaired nervous system[11, 16, 20]. FSCs can differentiate into virtually any type of neuron (as well as non-neuronal cells) when administered to patients with neurological diseases. This has been proven in multiple studies with labeled nuclei[11]. However, the engraftment rate of the transplanted cells is low (5%–20%), and is influenced by the method used to isolate stem cells from the fetal brain[19–20]. Another possible therapeutic role for FSCs is in preventing mitochondrial dysfunction and hence nerve cell degeneration[2]. It is also assumed that FSCs contain specific muscle markers and can express the dystrophin gene[12–14].

Based on these findings, this study was designed to evaluate changes in spirometry parameters after FSC therapy for grade 1 and II RF in ALS and MD patients.

2 Materials and methods

2.1 Subjects

The study involved 41 patients with ALS or MD complicated by RF. The cohort consisted of 19 patients (46.3%) with ALS aged 25–60 years (mean age, 39.4 ± 2.57 years), including 13 men (68.4%) and 6 women (31.6%), and 22 patients (53.7%) with MD aged 15–60 years (mean age, 36.3 ± 2.46 years), including 15 men (68.2%) and 7 women (31.8%).
The 41 patients were divided into 4 groups according to their underlying disease and degree of RF:

1. Group I: ALS patients with Grade I RF – 9 patients (22.0%).
2. Group II: ALS patients with Grade II RF – 10 patients (24.4%).
3. Group III: MD patients with Grade I RF – 11 patients (26.8%).
4. Group IV: MD patients with Grade II RF – 11 patients (26.8%).

MD was diagnosed on the basis of clinical findings, past history, electroneuromyography (ENMG) results that were suggestive of MD, laboratory test findings (abnormal alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) activity), genetic test results, and histological findings. ALS was confirmed on the basis of clinical and ENMG findings. The cervical form was diagnosed in 6 patients (31.6%), the cervicothoracic form in 5 patients (26.3%), and the bulbar form in 8 patients (42.1%). RF was diagnosed according to clinical symptoms (apnea, acrocyanosis, and a heart rate frequently exceeding 90 bpm), and measurements such as blood oxygen saturation (Grade I RF, 93–98%; Grade II RF, 86%–92%; Grade III RF, <75%), forced vital capacity (FVC) (Grade I RF, 70%–85%; Grade II RF, 50%–70%; Grade III RF, <50%), and forced expiratory volume in 1 second (FEV₁) (Grade I RF, 74%–55%; Grade II RF, 54%–35%; Grade III RF, <35%).

Lung ventilation capacity was measured using a BTL-08 Spirograph (2010) in accordance with the approved standards, and arterial oxyhemoglobin (SpO₂) was controlled using a pulse Oxymeter YX300 Armed (2011).

2.2 Stem cells

We used suspensions containing FSCs harvested from 5–9-week-old human fetuses. One suspension was made using stem cells from the fetal liver, while the other consisted of stem cells from fetal brain. The detailed protocol for making these suspensions has been previously published. Briefly, fetal material was harvested in the surgery room, in compliance with all aseptic and antiseptic requirements. After obtaining written informed consent all tissues were collected from the donor, mainly from the liver and brain tissue of 5–9-week-old human fetuses aborted for family planning reasons and found to have no developmental abnormalities or hemic infections. The tissue was then placed into sterile transport medium consisting of Hank’s Balanced Salt Solution and antibiotics (penicillin–streptomycin, 100 U–100 mg/ml) (Sigma Chemical Company, St. Louis, MO, USA). The tissues were aseptically separated and homogenized in Hank’s solution. The stem cell suspension was then filtered. Dimethyl sulfoxide 10% diluted in DMEM was used as a cryoprotectant. Cryopreservation of cell suspensions was performed in a controlled-rate freezer chamber pursuant to the selected program.

In order to ensure safety, both the donors and the ready-made stem cells in suspension from fetal liver were tested for the presence of bacteria, fungi, parasites and viruses such as human immunodeficiency virus 1 and 2, hepatitis B virus, hepatitis C virus, cytomegalovirus, herpes simplex virus 1 and 2, Epstein–Barr virus, rubella, syphilis (Treponema pallidum), toxoplasmosis (Toxoplasma gondii), Mycoplasma genitalium bacterium, Ureaplasma urealyticum, Chlamydia trachomatis, and Ureaplasma parvum.

Suspensions containing stem cells from fetal liver and brain were stored in liquid nitrogen at −196 °C in a properly arranged cryobank. Transplantation of the suspension containing cryopreserved fetal stem cells was preceded by an infusion of diphénylhydramine 10 mg and prednisone 30 mg on day 1 and a specially prepared solution on day 2. On day 1, stem cells from fetal liver were transplanted. A suspension containing cryopreserved stem cells was administered via an intravenous drip-feed in a volume of 1.75 ± 0.51 mL with a nucleated cell count of 58.74 × 10⁶/mL per transplantation. Fetal brain stem cells were subcutaneously administered on day 2 in a volume of 2.12 ± 0.49 mL with a nucleated cell count of 7.9 × 10⁶/mL per transplantation. The number of CD₃⁴⁺ cells was determined using flow cytomerometry (Becton Dickinson, Franklin Lakes, NJ, USA) with fluorescently tagged antibodies (Santa Cruz Biotechnology, Dallas, TX, USA).
All patients also underwent routine therapy including muscle supporting medicines (L-carnitine, vitamins, lipoic acid, amino acids, vasoactive medications, and biostimulators), and antioxidants in order to reduce muscle cell membrane damage.

Neurological evaluation, laboratory tests (ALT, AST, CPK, and LDH levels) and instrumental examinations were performed before FSCT, and 6 and 12 months after treatment. Before the FSCT, all patients or their legal representatives signed the informed consent. This study was approved by the local ethics committee and included in the public registry of the Ministry of Education and Science of Ukraine, Ukrainian Institute of Scientific, Technical and Economic Information. The study registration number is 0113U000957. Approval was obtained by the local ethics committee of Kyiv City Clinical Hospital for Accident and Emergency Care.

2.3 Statistical analysis

Average values and their standard deviations were calculated, and significant differences were determined using Statistika 6.0. Student’s T-criteria. Differences were regarded as statistically significant if $P < 0.05$.

3 Results

Objective parameters of respiration (FVC, FEV$_1$, and SpO$_2$) in ALS patients are presented in Table 1. After FSCT, the SpO$_2$ in ALS patients with Grade I RF (Group I) had increased by 1.8 ppm ($P < 0.05$) 6 months after treatment and by 4.0 ppm ($P < 0.05$) one year after treatment in comparison with baseline. The FVC had increased by 17.3 ppm ($P < 0.05$) and the FEV$_1$ had increased by 18.1 ppm ($P < 0.05$) in the same group 6 months after FSCT. An increase in respiratory volume was also apparent in Group II 12 months after treatment; the FVC was elevated by 18.5 ppm ($P < 0.05$) and the FEV$_1$ by 19.5 ppm ($P < 0.05$).

Thus, in combined treatment of ALS patients with Grade I RF, FSCs produced a positive clinical effect as demonstrated by an increase in respiratory volume. Furthermore, respiratory improvements were evident within 6 months of the start of treatment and were maintained one year after the treatment.

In ALS patients with Grade II RF (Group II), SpO$_2$ increased by 4.2 ppm ($P < 0.05$) over 6 months and by 5.2 ppm ($P < 0.05$) one year after treatment. The baseline respiratory volumes were significantly lower in these patients owing to the rapid progression of the disease, although despite this the FVC increased by 3.2 ppm ($P > 0.05$) and the FEV$_1$ by 2.1 ppm ($P > 0.05$) 6 months after FSCT, and within 12 months the FVC had increased by 4.7 ppm ($P > 0.05$) and the FEV$_1$ by 3.43 ppm ($P > 0.05$) in comparison with the baseline. Thus, FSCs tend to increase lung ventilation capacity and preserve respiratory capacity in these patients.

Spirometry findings and oxygen saturation levels of the MD patients are presented in Table 2. The most obvious effect of stem cell therapy was seen in MD patients with Grade I RF (Group III). FVC increased by 9.6 ppm ($P < 0.05$) and FEV$_1$ by 10.47 ppm ($P < 0.05$) over the 6 months following FSCT, and after 12 months they had increased by 10.64 ppm ($P < 0.05$) and 11.9 ppm ($P < 0.05$), respectively. Oxygen saturation levels within this group had also increased by 2.7 ppm ($P < 0.05$).

### Table 1 Spirometry findings and oxygen saturation in ALS patients

<table>
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<th>Group I</th>
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<th>Group II</th>
<th></th>
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<tr>
<td></td>
<td>Before FCST</td>
<td>6 months after FCST</td>
<td>12 months after FCST</td>
<td>Before FCST</td>
</tr>
<tr>
<td>FVC</td>
<td>72.36 ± 0.73</td>
<td>89.69 ± 1.12$^a$</td>
<td>90.86 ± 0.98$^a$</td>
<td>55.60 ± 1.06</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>73.12 ± 0.7</td>
<td>91.17 ± 1.08$^a$</td>
<td>92.58 ± 0.84$^a$</td>
<td>56.35 ± 1.3</td>
</tr>
<tr>
<td>SpO$_2$</td>
<td>93.66 ± 0.28</td>
<td>95.44 ± 0.33$^a$</td>
<td>97.66 ± 0.23$^a$</td>
<td>89.12 ± 0.3</td>
</tr>
</tbody>
</table>

**Note:** $^aP < 0.05$, statistically significant difference in values prior to therapy and 6 months after FSCT; $^bP < 0.05$, statistically significant difference in values prior to treatment and 12 months after FSCT; ALS, amyotrophic lateral sclerosis; FCST, fetal stem cell treatment; FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1 second; SpO$_2$, arterial oxyhemoglobin saturation.

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Table 2  Spirometry findings and oxygen saturation levels in MD patients

<table>
<thead>
<tr>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td></td>
<td>Before FCST</td>
</tr>
<tr>
<td>FVC</td>
<td>82.47 ± 0.69</td>
</tr>
<tr>
<td>FEV₁</td>
<td>82.99 ± 0.63</td>
</tr>
<tr>
<td>SpO₂</td>
<td>94.18 ± 0.53</td>
</tr>
</tbody>
</table>

Note: *P < 0.05, statistically significant difference between values prior to treatment and 6 months after FSCT; †P < 0.05, statistically significant difference between values prior to therapy and 12 months after FSCT; FCST, fetal stem cell treatment; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second; MD, muscular dystrophy; SpO₂, arterial oxyhemoglobin saturation.

over the 6 months following therapy, and by 3.4 ppm (P < 0.05) over 12 months. Thus, in the combined treatment of Grade I RF in MD, FSCs significantly improved respiration with respect to FVC and FEV₁. In these patients, normalization of breathing was possible within 6 months after the start of treatment and was maintained through the subsequent 6 months.

Improvements were also apparent in MD patients (Group IV) with Grade II RF. Six months after FSCT, the FCV had increased by 3.2 ppm (P > 0.05) and FEV₁ by 2.8 ppm (P > 0.05). Twelve months after therapy the increase in respiratory capacity was significantly higher than the baseline: FEV increased by 13.5 ppm (P < 0.05) and FEV₁ by 12 ppm (P < 0.05). The post-FSCT SpO₂ increased by 5.4 ppm (P < 0.05) over 6 months and by 6.8 ppm (P < 0.05) over 12 months. Thus, after FSCT, MD patients with Grade II RF generally had improved respiration through a significant increase in respiratory capacity, which allowed the patient to be downgraded to Grade I RF.

4 Discussion

The experimental application of FSCs continues to be debated, and is not supported in the USA and some European countries. However, in many countries, including Ukraine, this method of therapy can be used experimentally provided that consent for abortion and donation of the fetal material is obtained based on legal, moral, and ethical principles. In view of very promising clinical results using FSCs, which are able to differentiate into specialized functional cells, including various muscular tissues, their routine use may now be justifiable.

In our study, the improvements in the patient’s condition, including their respiratory function, were probably due to the distribution of multipotent stem cells throughout the body, allowing them to repair defects both locally and systemically. This is seen in MD patients in general, and Duchenne MD in particular[14]. Moreover, stem cells could promote muscle regeneration through their properties of self-renewal and differentiation, as demonstrated in several pre-clinical models[23]. The rationale for the use of FSCs derived from liver and brain tissue, administered on different days, is based on our previous experience[24]. In Duchenne MD, FSCs could exert a beneficial effect based not only on their ability to differentiate into functional cells, but also through a trophic paracrine effect, as they can secrete diffusible growth factors. It is also possible that transplanted FSCs could restore dystrophin synthesis.

In addition to improvements in breathing capacity, FSCT helped improve the patient’s physical and emotional state, with improved self-confidence and social functioning. Consequently, patients receiving FSC treatment considered that their overall quality of life had greatly improved.

5 Conclusions

5.1 In combined treatment of Grade I RF in ALS
patients, FSCT resulted in respiratory improvement as early as 6 months after treatment, and this was maintained over the next 6 months. Although there was no significant respiratory capacity improvement in ALS patients with Grade II RF, their ventilation capacity tended to increase.

5.2 In combined treatment of MD patients with Grade I RF, FSCT resulted in respiratory function normalization within 6 months. In MD patients with Grade II RF, treatment resulted in significant FVC and FEV, increases within 6 months and downgrading to Grade I RF within a year.

Conflict of interests

The paper is intended to be an original paper; all authors of the manuscript, except DS, are members of Cell Therapy Center EmCell, Kyiv, Ukraine. The authors have approved the manuscript and agree to its submission. There are no matters that constitute a conflict of interest among the authors who contributed to this manuscript.

References


