Clinical Safety in Using Unmatched Allogeneic Umbilical Cord Blood Mononuclear Cells Transplantations in Non-Haematopoietic Degenerative Conditions

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Abstract

Aim: Evaluation of safety in using unmatched human allogeneic umbilical cord blood cells for therapeutic use in individuals with non-haematopoietic degenerative conditions.

Background: The historical data and several recent immunological arguments suggest the therapeutic use of allogeneic Cord Blood Mononuclear Cells (CBMNCs), as these cells do not elicit immune response. Customarily, HLA matched cord blood MNCs are used along with prolonged immunosuppression in treatment of haematological conditions. Lately, unmatched CBMNCs are widely used in case of unavailability of HLA matched cord blood. There have been suggestions for using unmatched allogeneic cord blood MNCs for degenerative conditions without an immunoconditioning regimen.

Method: 49 patients with non-haematopoietic degenerative conditions were treated with HLA-unmatched allogeneic hUCB MNCs. Intrathecal/I.V injections (1-2 million cells/kg body weight) were given. Clinical, biochemical and haematological adverse events were evaluated.

Results: The haematological and biochemical parameters showed no major deviation from the normal. Clinically, no acute adverse effects or GVHD were observed with the used dosage.

Conclusion: This study supports/suggests clinical safety in therapeutic medical use of unmatched allogeneic CBMNCs when used at low dosage in non-haematopoietic degenerative conditions.

Keywords: Degenerative conditions, Graft-Versus Host Disease (GVHD), Human Umbilical Cord Blood Mononuclear cells (hUCB MNCs), Regenerative Medicine, Stem cell Therapy

Introduction

The human umbilical cord blood mononuclear cells (hUCB-MNCs) are heterogeneous population of hematopoietic and mesenchymal stem cells,
endothelial progenitor cells, unrestricted somatic stem cells (USSC) and very small embryonic-like stem cells (VSEL).[1, 2, 3] These cells are multipotent, i.e. differentiate into chondrocyte, adipocyte, osteocyte, myocyte, hepatic cells and neuron-like cells and exhibit self-renewability.[4] [5] The conventional medical use of cord blood cells is limited to hematopoietic reconstitution. [6] In such cases ablation of recipient marrow is required to eradicate the endogenous stem cell compartment prior to transplantation of CBMNCs followed by prolonged immunosuppression to prevent GVHD (Graft-Versus-Host-Disease). [6] For non-hematopoietic applications the therapeutic activities of the cord blood are believed to be mediated by growth factor secretion (paracrine effect). [7, 8] Therefore permanent graft survival is not essential. There are reports of long term benefits in chronic degenerative diseases. [9] It is likely that this beneficial effect may be derived on account of concurrent application of immunonaive MSCs and VSECs that are present, though in small percentage, in hUCB. Hence, the use of non-matched, allogeneic cells may be acceptable. The major barrier to this approach is the theoretical fear of inducing acute adverse reactions and GVHD.

The previous studies indicate the possibility that, non-matched allogeneic cord blood cells do not elicit GVHD [10, 11] These cells have shown no adverse immunological effects and are found to be immunonaive in allogeneic experimental models. [12] Our proof of concept study is designed to confirm these observations in different degenerative clinical conditions.

It is reported that, hUCB-MNCs transplantation into animals and humans showed significant functional improvements. [13, 14] CB-MNCs have shown significant positive results in non-haematological studies in regenerative medicine. In medical practice, unmatched cord blood stem cells without immune suppression have widely been used for treatment of degenerative conditions, by several other groups. [10, 15] The present study evaluates clinical, haematological and biochemical parameters of 49 patients treated with allogeneic cord blood mononuclear cells. This study asserts safety in using allogenic UCBMNCs for regenerative and therapeutic use.

Methods

The procurement of cord blood and the clinical trial was approved by the IEC (Institutional Ethics Committee) and IC-SCRT (Institutional Committee for Stem Cell Research and Therapy). These were registered for clinical trial with TRI (Clinical Trial Registry of India). The research was conducted as per the ‘Ethics Guidelines’ stipulated by Indian Council of Medical Research. The present study was conducted at Samanvaya Trust, Vadodara from March 2010 to June 2013.

Collection of Human Umbilical Cord Blood

Human UCB samples were collected using ACD blood bags (MITRA JML) at birth, with prior consent of the mother, from term or preterm Caesarean section deliveries. The mothers were tested negative for HIV, HBV, HCV and Syphilis. The UCB samples were then processed immediately to get the mononuclear cells.

Mononuclear Cells Separation

Umbilical Cord Blood (60~120 ml) was collected as mentioned before following the sterile procurement guidelines for cord blood. This was centrifuged at 2000 rpm at 18°C for 20 minutes to obtain a buffy coat layer. The buffy coat was collected in PBS in the ratio 2:1 and was layered on 15 ml Hi Sep™ and centrifuged at 3500 rpm for 35 minutes. The monocuclear cells obtained were collected and washed twice with PBS. The pellet obtained was re-suspended with 50 ml Isolyte-M (dextrose) and centrifuged twice at 2000 rpm at 18°C for 10 minutes. Contaminating erythrocytes were lysed with distilled water. To achieve this, the pellet (containing MNCs) was treated with 1 ml of sterile distilled water and gently mixed to expose RBCs to distilled water for 30 seconds. Immediately, 1 ml of 2xPBS was added for the MNCs to stabilize and this was centrifuged twice at 1200 rpm for 10 minutes. The pellet was suspended in 1xPBS. After cell counting and cell viability test, fresh cell suspension
in 5 ml injectable Isolyte-M was used for administration.

**Patient Selection**

The following patient selection criteria were considered: No prior history of severe allergic reactions; no history of malignancy; no active infections (seronegative for HIV, HBV, HCV and syphilis); no active cardiac, pulmonary, renal, hepatic or gastrointestinal disease; no coagulopathy or any other contraindication for lumbar puncture; no severe psychiatric disorder and they had no symptoms suggestive of immunodeficiency disease or condition. Selected age range for patients was 03 to 80 years and the male: female ratio was 1.9:1 (31 males, 16 females). In terms of diagnosis, 3 patients had muscular dystrophy, 2 patients had osteoarthritis, 4 patients had brain stroke, 1 patient had autism and 1 had mental retardation, 2 patients had neurological residual symptoms following meningitis, 9 patients had cerebral palsy, 14 patients had spinal cord injury. 10 patients had other diagnoses. (Table 1) Prior to the treatment, informed consent from the patients and the approval of therapy was obtained.

### Table 1. Degenerative conditions in patients

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine injury</td>
<td>14</td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td>09</td>
</tr>
<tr>
<td>Brain stroke/disorder</td>
<td>04</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>03</td>
</tr>
<tr>
<td>Viral meningitis</td>
<td>02</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>02</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>02</td>
</tr>
<tr>
<td>Autism</td>
<td>01</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>01</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>01</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
</tr>
</tbody>
</table>

**MNC Administration in Patients**

1-2 million hUCB-MNCs/kg of body weight were given to patients by various mode of administration. The dose chosen for this study was selected as per previously reported safe CBMNC dosages, by other workers in various clinical transplantation set ups. [11][16][17] 5 patients were given a repeat dose of MNC twice or thrice over a period of 2 to 6 weeks. Cord blood derived MNCs were injected in combination with 10-15 ml of autologous Platelets Rich Plasma (PRP). Depending on the age and the body weight of the patient, hydrocortisone, antibiotics, metronidazole and antihistamines were given prior to CBMNC administration. The follow up was done on the patients for clinical and biochemical parameters after the treatment.

**Results**

No adverse clinical reactions occurred; except mild fever and headache lasting for 1-2 days, while 3 patients had prolonged mild fever up to a week. Their clinical investigations did not suggest any infection, i.e., no change in total WBC count and no elevations in CRP levels were observed. 28 out of 49 patients received the hUCB more than once at the interval between 3 to 36 weeks. There was no evidence of sensitization. Mild skin rash was observed in one patient, 5 days after MNC administration, but it was self-limiting and subsided within 4-5 days. No allergic reactions / anaphylaxis occurred. Though used as a precaution once before the administration of hUCBMN cells, anti-allergic drugs and corticosteroids were not needed later. Only analgesics and anti-pyretics were used in those patients who had fever or headache. No GVHD or serious adverse effects were observed. Haematological and biochemical parameters remained within normal range. This suggests that no immune response occurred. Table 2 shows the incidence of adverse events in patients per administration of MNCs. Table 3 below represents the biochemical and haematological parameters observed in patients.

These values fall in the normal range before and after the treatment and are represented as average range. There were no anaphylactic or allergic manifestations in patients who were given a repeat dose of CBMNCs.
Table 2. Analysis of adverse events

<table>
<thead>
<tr>
<th>Type of adverse event</th>
<th>No. of patients affected</th>
<th>Percentage of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>03/49</td>
<td>6.12%</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>04/49</td>
<td>8.16%</td>
</tr>
<tr>
<td>Shivering</td>
<td>01/49</td>
<td>2.04%</td>
</tr>
<tr>
<td>Limb pain</td>
<td>01/49</td>
<td>2.04%</td>
</tr>
<tr>
<td>Skin rash</td>
<td>01/49</td>
<td>2.04%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>01/49</td>
<td>2.04%</td>
</tr>
</tbody>
</table>

Table 3. Haematological and Biochemical parameters observed in patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observed range</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>13.02 ± 3.94</td>
<td>11.0-16.0</td>
</tr>
<tr>
<td>TC (c/mm)</td>
<td>6460 ± 72.28</td>
<td>3800-9800</td>
</tr>
<tr>
<td>Platelets (x10^7/μl)</td>
<td>190 ± 12.91</td>
<td>180-350</td>
</tr>
<tr>
<td>RBC (x10^12/l)</td>
<td>4.745 ± 2.46</td>
<td>3.50-5.50</td>
</tr>
<tr>
<td>Random Glucose (mg/dl)</td>
<td>102.2 ± 0.001</td>
<td>&lt;160</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.74 ± 0.79</td>
<td>0.10-1.40</td>
</tr>
<tr>
<td>SGPT (IU/l)</td>
<td>30.6 ± 0.16</td>
<td>5.0-40.0</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>1.12± 0.12</td>
<td>Up to 1.2</td>
</tr>
</tbody>
</table>

Discussion

Unrelated unmatched UCBMNCs are widely used as an alternative source of haematopoietic cells for the patients lacking a HLA-matched donor. Also, MSCs are broadly used for degenerative diseases. [9, 13] UCB has many advantages over bone marrow or mobilized haematopoietic stem cells from peripheral blood of volunteer donors. These advantages are: rapid availability, absence of risk for the donor, and minimal incidence of acute GVHD. [18]

The possibility of using un-matched, allogeneic cord blood cells for regenerative applications without having to do complete or partial bone marrow ablation would overcome several substantial hurdles in stem cell therapies in the future. Although cord blood derived cells are superior to bone marrow in terms of growth factor production ability, multipotency, and immune modulating activity [19], [20] their use has been limited to autologous sources for regenerative applications. Until now, the use of allogeneic CBMNCs for degenerative conditions had to be followed by immune suppressive therapy. The current study investigates the ‘safety’ of allogeneic CBMNCs for their use in regenerative applications without the need of prolonged immune suppression.

Here, all the 49 patients were administered allogeneic CBMNCs in absence of prolonged immunosuppressive drugs. No serious acute adverse effects were observed. The minor adverse effects that were observed were development of skin rash in one patient (2.04%), 4 patients (8.16%) had pyrexia (fever not exceeding 102 °F), headache was observed in 3 patients (6.12%) and shivering in 1 patient (2.04%). Headache was observed in patients who were given intrathecal injections. The symptoms like skin rash
resolved spontaneously, while fever and headache subsided after treatment with antipyretic and analgesics. No aggressive intervention was required.

The method for removal of RBC was developed in house at TPC. The exposure to DW brings about RBC lysis and the centrifugation at low speed settles the MNCs. Based on their sedimentation coefficient RBC antigens remain in the supernatant and are removed in the subsequent steps. Moll et al. have concluded that the ABO antigens do not hamper the therapeutic efficiency of mesenchymal cells. [21]

Here, MNC suspension devoid of RBCs was confirmed by microscopic examination and MNCs were tested positive for viability after the lysis treatment.

In summary, the present study suggests ‘safety’ in using allogeneic cord blood MNCs for the treatment of degenerative diseases in patients without hematopoietic or immune deficiency diseases. Although, it suggests that there is no need of prolonged immunosuppression following therapy with allogeneic CBMNCs, we recommend use of hydrocortisone, metronidazole and antihistamines prior to CBMNCs administration. This is to overcome acute mild adverse effects that were encountered during the present study. This observation is in consensus with the study done by Yang et.al. [11]

This study will help in clinical translation of allogeneic CBMNC transplantations at the general and recommended dosages used here, especially in unmet degenerative medical conditions. This report strongly appeals to reconsider the apprehensive approach in stem cell based therapy of degenerative disorders and overcome the theoretical fear of probable adverse effects.

Declaration by authors

The authors declare that there was no conflict of interests. The study was not funded by any private or government funding body.

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References


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