TRANSPLANTATION OF MARROW DERIVED MESENCHYMAL STEM CELLS AND PLATELET RICH PLASMA DURING DISTRACTION OSTEOGENESIS
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Introduction

Distraction osteogenesis is a biological treatment and has been successfully used for limb lengthening, but the periods of external fixation is long which results in higher rates of complications. Decreasing the treatment period by accelerating new bone formation of the distracted callus could reduce these complications.

Bone marrow derived mesenchymal stem cells (MSCs) can be directed towards the osteogenic lineage in vitro if cultured with osteogenic supplements. Using the rat limb lengthening model, we have previously demonstrated that transplantation of marrow derived osteoblast-like cells with collagen gel into the distracted callus promoted new bone formation and shortened the consolidation period1. Platelet rich plasma (PRP), which is known to contain several growth factors, coagulates immediately by a minute introduction of calcium and thrombin.

We performed a new cell therapy during distraction osteogenesis using culture expanded MSCs and autologous PRP to shorten the treatment period and reduce associated complications.

Patients and Methods

After informed consent was obtained from all individuals prior to surgery, 24 limb lengthenings (12 femora and 12 tibiae) with transplantation of MSCs and PRP were performed in 13 patients (short stature : 10, limb length discrepancy : 3). All patients were followed up until removal of the pins.

During the surgery, approximately 40 ml of bone marrow aspirates were collected from the iliac crest and mononuclear cell fractions were plated in DMEM supplemented with osteogenic supplements. Adherent cells were cultured for nearly three weeks for transplantation. For evaluation of osteoblastic differentiation of MSCs, the concentration of bone specific alkaline phosphatase (BAP) and carboxy-terminal propeptide of type I collagen (PICP) in the culture medium was measured in each passage. PRP was processed by two-staged centrifugation from approximately 200 ml of venous blood, which was drawn within 48 hours before transplantation for fear of a decline in platelets function. Under X-ray guidance, two spiral needles were inserted at the center of the distracted callus face to face with each tip, culture expanded MSCs and autologous PRP were injected into the distracted callus with the thrombin calcium mixture so that the PRP gel might develop within the injected site.

Result

The third-passaged (P3) cells with the average of 3.1×10⁷ were used for transplantation. The BAP activity within the culture medium was the highest in the P2 cells and maintained at all passages (Fig. 1). On the other hands, a gradual increase was observed in the secretion of PICP during passaging (Fig. 1). The average amount of 198ml whole blood was drawn and PRP was processed at the average of 12 ml. The serum platelet concentration was averaged of 2.39×10⁷/UL in a whole blood, while platelets reached an average concentration of 27.7×10⁷/UL in a processed PRP, which was 11.5 times the serum level.

The average age at the surgery and the amount of lengthening were 16 years and 7.6 cm. The healing index ranged from 18.2 to 37.6 days/cm with an average of 28.8 days/cm (Table 1). For clinical assessment of the treatment, nine achondroplasia (ACH) patients who underwent 32 limb lengthenings without MSCs transplantation were compared to eight ACH patients (16 legs) treated with MSCs and PRP transplantation. The average age at the surgery and the amount of lengthening were similar in both groups, while, the healing index was significantly smaller in the ACH patients with MSCs and PRP transplantation (Table 2).

Discussion

We showed that transplantation of MSCs and PRP could shorten the treatment period by acceleration of bone regeneration during distraction osteogenesis2. Autologous cell therapy for bone regeneration by combination of MSCs and PRP has many advantages in clinical feasibility. First, MSCs can be expanded ex vivo and manipulated into osteoblastic lineage. Second, the treatment is safe with minimal side effect because both MSCs and PRP are autologous which are nontoxic and nonimmunoreactive. Third, transplantation of MSCs by injection is less invasive resulting in low risks of infection. The technique we proposed may be applicable for the repair of bone defects, and could be a useful alternative to allogeneic or autologous bone grafts, which appear to be safe, minimally invasive, and easy to perform, with great potential in clinical applications.

![Graph showing BAP and PICP activities in the culture medium](image)

**Table 1** Overall results of MSCs and PRP transplantation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age</th>
<th>Amount</th>
<th>Healing index</th>
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<tbody>
<tr>
<td>Femur</td>
<td>32</td>
<td>16.3</td>
<td>8.8 cm</td>
<td>24.3 days/cm</td>
</tr>
<tr>
<td>Tibia</td>
<td>32</td>
<td>15.1</td>
<td>7.2 cm</td>
<td>33.3 days/cm</td>
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<tr>
<td>Total</td>
<td>64</td>
<td>16.0</td>
<td>7.6 cm</td>
<td>28.8 days/cm</td>
</tr>
</tbody>
</table>

**Table 2** Comparison of healing index in achondroplasia patients with and without MSCs transplantation

REFERENCES

2 Kitoh H et al, Bone 35:892-898, 2004