Introduction

Orthopaedic patients who need substitute for a large bone defect have increased especially in revision arthroplasty or in tumor surgery. Now, three options as the substitute are available; autogenous, allogenic or artificial bone. Autogenous bone tissue has high quality for bone induction and bone conduction, and autograft causes no disease transmission unlike allograft from the donor to the recipient. However the amount of autogenous bone tissue is limited and not enough for a large bone defect. One solution for the quantity problem is to induce required bone tissue by osteoinductive growth factors.

The substitute for a large bone defect often needs supporting some weight bearing. There may be a possibility that osteoinductive growth factors with a carrier function like free autogenous bone tissue. However, free or devascularized bone graft would be collapsed against the weight bearing. Autogenous bone tissue that has both conformity to the defect and vascularity would be the good substitute.

Bone morphogenetic protein-2 (BMP-2) is well known to have a capability to induce ectopic bone formation in muscle tissue. Our idea is to induce morphologically controlled bone tissue in muscle, and to use the induced bone as muscle pedicled autograft. We chose beta tri-calcium phosphate (βTCP) as a carrier that has hardness and can be absorbed. First purpose of our study is to examine morphologically controlled induction of bone in muscle using βTCP as a carrier for BMP-2. Secondary, viability of the induced bone in a pedicled form of muscle tissue was investigated.

Method

Human recombinant BMP-2 (hrBMP-2) was provided by Yamanouchi Pharmaceutical Co., Ltd. βTCP was provided by Olympus Optical Co., Ltd.

1st experiment: Adult Japanese white rabbits (approximately 3 kg) were used. Under anesthesia, fifty µg of hrBMP-2 with a carrier was inoculated into the hip abductor muscle. As a carrier, 7.5x5x3 mm³ of a rectangular parallelepiped of βTCP was used. Before inoculation, hrBMP-2 solution was put on the carrier, and the carrier was on ice for 30 minutes. Five or ten weeks after operation, the inoculated muscle was harvested. Bone formation was evaluated histologically in three characters including circumferential, inside bone formation and rectangular structure. More than a half, less than a half or none of circumferential bone formation was evaluated as 2, 1, or 0 points, respectively. More than ten islands of new bone formation inside the carrier, less than ten or none were evaluated as 2, 1, or 0 points, respectively. More than three rectangular corners of the induced bone, less than three or none were observed, it was evaluated as 2, 1 or 0 points, respectively.

2nd experiment: Fifty µg of hrBMP-2 with βTCP was inoculated in the hip abductor muscle under anesthesia. Five weeks after operation, muscle tissue around the induced bone tissue was incised leaving just proximal part as a pedicle (Fig. 1). The pedicled muscle was wrapped with vinyl in order to prevent revascularization from the incised portion. Two or four weeks after the 2nd operation, the pedicled muscle that had a carrier was harvested, and examined radiographically and histologically.

Results

1st experiment (Table 1): Five weeks after inoculation, intramuscular bone formation was all observed, and the form of the induce bone tissue was equal to the form of the carrier. Bone formation was observed inside the carrier as well as around it. Ten weeks after inoculation, the induced bone tissue was partly absorbed.

2nd experiment: Two weeks after the 2nd operation (n=6), ectopic bone formation was observed in the pedicled muscle, and the size and structure were maintained (Fig. 2). Histological study showed that the bone tissue had rich vascularity and no empty lacunae indicating that the induced bone tissue was alive and well vascularized (Fig. 3). Four weeks after the 2nd operation (n=6), the bone tissue was partly absorbed although it was alive histologically. No ectopic bone formation was observed in control muscle (βTCP only, n=4, 4).

Discussion and Conclusion

Inoculation of BMP-2 with beta TCP in muscle tissue induced morphologically controlled bone. And this induced intramuscular bone could be moved as muscle pedicled, or vascularized bone tissue. This system would enable bone banking in order to use large vascularized and morphologically-controlled autogenous bone that can support weight bearing as a substitute for the bone defect.

Table 1. Histological evaluation of induced bone tissue

<table>
<thead>
<tr>
<th>Bone formation</th>
<th>RhBMP+βTCP (n=4)</th>
<th>βTCP only (n=4)</th>
<th>RhBMP+βTCP (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumferential</td>
<td>2</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>Inside</td>
<td>2</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Corners</td>
<td>2</td>
<td>0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Fig. 1. A: a pedicled muscle that had ectopic bone inside (an arrow). B: The pedicled muscle was wrapped with vinyl.

Fig. 2. Radiographs of ectopic bone tissue. A: two weeks after the 2nd operation B: four weeks.

Fig. 3. Histology of induced ectopic bone tissue two weeks after the 2nd operation. Many osteoblasts (arrows) were lined on TCP, and newly formed bone (*) was observed. Many capillary vessels were formed.