ORIGINAL ARTICLE

Bone Formation Induced by Recombinant Human BMP-2 Combined with Bioabsorbable Material at Subperiosteal Sites

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SYNOPSIS

The purpose of this study was to evaluate the effect of bone formation induced by recombinant human BMP-2 (rhBMP-2) combined with bioabsorbable material Kurabio®AG (AG) at palatal subperiosteal sites in Wistar rats. AG was freeze-dried alginate gel dressing.

Experimental sites were divided into three groups according to the implants: BMP group (rhBMP-2 combined with AG), AG group (AG alone) and Control group.

In every group, infected region or atypical cell proliferation was not observed, and new bone formation was observed at 6 weeks after implantation. Thickness of new bone (TNB) of BMP group was significantly higher than that of AG and Control groups. There was no significant difference in TNB between AG and Control groups.

These results suggest that rhBMP-2 combined with AG have the ability to promote bone formation, although AG alone do not have any effect on bone formation.

Key words: bone formation, rhBMP-2, bioabsorbable material

INTRODUCTION

It has been suggested that recombinant human BMP-2 (rhBMP-2) enhance the healing of bone defects and the periodontal regeneration in previous experimental studies1-13. And it has been reported that the palatal subperiosteal implantation of rhBMP-2 results in new bone continuous with the original bone1,5,6.

For the application of rhBMP-2 in vivo, rhBMP-2 require a suitable scaffold: fibrous collagen membrane, polylactate-polyglycolate-copolymer /gelatine sponge, collagen sponge, collagen gel, and so on. It is important to select the appropriate scaffold for BMP. Recently, a bioabsorbable material Kurabio®AG (AG), which is freeze-dried alginate gel dressing, has used as the clinical wound
healing material. It is unknown whether AG is a suitable scaffold for rhBMP-2 to induce bone formation.

The purpose of this study was to evaluate the effect of bone formation induced by rhBMP-2 combined with AG at palatal subperiosteal sites in Wistar rats.

MATERIALS AND METHODS

1. Animals

Six male Wister rats (body weight 308 ± 12g) were used in this experiment. All procedures involving rats were performed in compliance with guidelines for the care and use of laboratory animals of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

2. Combination of rhBMP2 with bioabsorbable material

RhBMP-2 (Astellas Pharmaceutical Co., Ltd, Tokyo, Japan) was dissolved in LF6 buffer (5 mM sodium glutamate, 2.5% glycine, 0.5% sucrose, 0.01% Tween 80, pH 4.5). AG (Kuraray Medical Co., Ltd, Tokyo, Japan) which was freeze-dried alginate gel dressing, was cut into 4.0 × 0.5 × 1.0 mm pieces, and then rhBMP-2 (2.0µg) was dropped onto AG.

3. Implantation

Bilateral palatal grooves at palatal subperiosteal sites were used as the experimental sites (two sites of every rat). These sites (n=12) were divided into three groups according to the implants: BMP group (rhBMP-2 combined with AG), AG group (AG alone) and Control group.

Surgical procedures, following the method of previous studies, were performed. Sulcular incisions were made at the palatal side of bilateral upper molars, and an incision crossing the palate was made posterior to the anterior palatal foramina. A full-thickness flap was raised backward from the cross incision on the left side of the palatal bone.

Nylon fibers were placed on the palatal bone surface as markers to distinguish new bone from original bone. The implant was implanted over the nylon fibers. The animals were sacrificed 6 weeks after surgery.

4. Histological procedures and histometric evaluations

Tissue blocks including palate and molar teeth were fixed. Fixed tissue blocks were demineralized, and were then embedded in paraffin. Serial sections 5-µm thick were cut in the frontal plane and were stained with hematoxylin and eosin.

The distance between the most nasal position of the nylon fibers and the new palatal bone surface was measured as the thickness of new bone, using light microscopy and a standard grid.

The value of thickness of new bone (TNB) at each implantation site was calculated by averaging the thickness of new bone of 5 sections, including the distopalatal root of the first molar, at intervals of 100µm.

5. Statistical analysis

The Tukey-Kramer test was used for comparisons of TNB among each group.

RESULTS

1. Histological observation

Infected regions or atypical cell proliferation were not observed in any of the observed specimens. All implants had disappeared, leaving the nylon fibers as markers distinguishing new bone from original bone. In most of the specimens, the nylon fibers were surrounded by bone tissue. The new bone was continuous with the original bone in all specimens (Fig.1).
Fig. 1 Photomicrographs of (a) BMP, (b) AG and (c) Control groups (hematoxylin and eosin stain). (bar = 100 μm)

The nylon fibers (arrowhead) in the specimens were surrounded by bone tissue at 6 weeks after implantation. The new bone (NB), distinguished by the nylon fibers, was continuous with the original bone (OB).

**Table.1** Thickness of new bone (TNB) of every group

<table>
<thead>
<tr>
<th>Group</th>
<th>BMP</th>
<th>AG</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNB (μm)</td>
<td>131 ± 42</td>
<td>65 ± 25</td>
<td>61 ± 22</td>
</tr>
</tbody>
</table>

All values are mean ± SD. (n=4)

Same letters indicate no statistical differences according to Tukey-Kramer test (P>0.05).

2. Histometric analysis

TNB of every group is shown in Table.1. TNB of BMP group was significantly higher than that of AG and Control groups. On the other hand, there was no significant difference in TNB between AG and Control groups.

DISCUSSION

The aim of the present study was to evaluate the effect of bone formation induced by rhBMP-2 combined with AG at palatal subperiosteal sites in male Wistar rats.

In this study, palatal subperiosteal
sites were used for implantation. Because it has been reported that the palatal subperiosteal implantation of rhBMP-2 results in new bone continuous with the original bone\(^4,5,6\). To evaluate the new bone histometrically, it is necessary to distinguish the new bone from the original bone in histological observations. The nylon fibers were chosen as markers, and used to put on the original bone surface at operation\(^4,5,6\).

In this study, these fibers appeared to be effective markers for the histometric evaluation of the new bone continuous with the original bone in the decalcified specimens.

It is important to select the appropriate scaffold for BMP. The desirable scaffold should satisfy the following criteria: 1) it ensure adequate retention and release of BMP, 2) have no inhibitory effects on bone formation, 3) be biodegradable, and 4) minimize immunogenicity.

An alginate-based gel material AG, was developed by Kuraray Medical Co., Ltd, (Tokyo, Japan), currently has used in the treatment of various wounds including bedsores, ulcers, burns and injuries. The freeze-dried alginate gel dressing shows no cytotoxic effects on fibroblasts, and has the possibility to cause the derivation of tissue\(^14,15\). It is unknown whether this material is a suitable scaffold for rhBMP-2 to induce bone formation. Therefore, we decided to use AG as the scaffold for rhBMP-2 in this study.

For the precise histometric evaluation of new bone, an adequate postoperative observation period is necessary. If the observation period is too short, histometric evaluation of the new bone can not be performed, because further bone formation might occur until the implanted material disappears completely. All implants had disappeared, and new bone formation was observed, which was continuous with the original bone in all specimens. In this study, 6 weeks seemed to be adequate postoperative observation.

Infected regions or atypical cell proliferation were not observed. It seemed that implantation of rhBMP-2 combined with AG, or AG alone was safety and harmless material.

TNB of BMP group was significantly higher than that of AG and Control groups. This indicated that the implantation of rhBMP-2 combined with AG was capable of stimulating bone formation. On the other hand, there was no significant difference in TNB between AG and Control groups. It seemed that the implantation of AG alone did not have any effect on bone formation.

In conclusion, the present results suggest that at palatal subperiosteal sites, rhBMP-2 combined with AG have the ability to promote bone formation, although AG alone do not have any effect on bone formation.

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REFERENCES


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