Effect of BMP-7 on Bone Regeneration by Interconnected-Porous Calcium Hydroxyapatite Ceramics Composite in Canine Lumbar Posterolateral Fusion Model

+1Nanno, K; 1Oshima, K; 1Sagiysku K; 1Hashimoto, N; 1Yoshikawa, H; 2Myoui, A
1Department of Orthopedics, Osaka University Graduate School of Medicine, Osaka, Japan
2Medical Center for Translational Research, Osaka University Hospital, Osaka, Japan
nanno@ort.med.osaka-u.ac.jp

Introduction
Bone morphogenetic proteins (BMPs) are biologically active molecules capable of inducing new bone formation and have been approved for clinical use in lumbar spinal fusion and other orthopedic conditions. However, an ideal drug delivery system (DDS) that can potentiate their bone-inducing ability is still strongly demanded because of the high cost of BMPs.

In this study, we examined the potential of interconnected-porous calcium hydroxyapatite ceramics (IP-CHA) as a DDS for BMPs. We used a canine lumbar posterolateral fusion model to evaluate the bone-regenerating efficacy of rhBMP-7/IP-CHA composite.

Methods
Preparation of implants
Cubic blocks of IP-CHA (10mm X 10mm X 10mm; total volume: 1000 mm³, pore space: approximately 700 mm³) were provided by Covalent Material Co. LTD. (Kanagawa, Japan). IP-CHA has a well-organized interconnected structure with total porosity of 70-75% and average interconnection channels diameter of 40μm. Theoretically, more than 90% of the pores are connected one another by channels with a diameter greater than 10μm, allowing tissue invasion from pore to pore.

Recombinant human Bone Morphogenetic Protein-7 (BMP-7) in a lyophilized 5% lactose formulation was provided by Stryker Biotech (Hopkinton, MA, USA). BMP-7 was dissolved in 60% of ethanol, and the solution containing 0.5mg of BMP-7 was dripped onto a cubic block of IP-CHA. After ethanol was removed by evaporation, the cubic block was then crushed into pieces of approximately 3-5mm in size.

Animal experiment
Nine male, pure-bred beagles aged 18-19 months weighing 9-13 kg were used in the study. Each animal was placed in a prone position and posterior cortices of the L4-L5 transverse processes were decorticated bilaterally using a high-speed burr until punctate bleeding occurs. The implants were placed into the right and left posterolateral gutters in direct apposition with each of the transverse processes, spanning the intertransverse space. The animals were divided into the three groups as shown below (three animals per group). Autologous tricortical iliac bone was harvested from the same animal and prepared as morselized corticocancellous chips for re-implantation at the graft site. The animals were kept in accordance with our institutional guidelines for care and use of laboratory animals. 8 weeks after implantation, these animals were sacrificed and the spinal columns were harvested together with surrounding tissues for further examinations.

Radiographic examination
Fusion sites were analyzed at 2, 4, 6, and 8 weeks after surgery using plain radiographs. 8 weeks after surgery, Micro-CT was also performed using Skyscan 1076 (Skyscan, Kontich, Belgium) (Fig.1).

Bone mineral density (BMD)
BMD was determined at 2, 4, 6, and 8 weeks after surgery by dual-energy X-ray absorptiometry (DXA) using an animal densitometer (Discovery-A, Hologic Inc., Bedford, MA, USA) (Fig.2).

Manul palpation
The harvested lumbar spines were manually palpated by stressing flexion and extension at the fusion site. Specimens were determined to be fused when no significant motion was observed (Table 1).

Histological examination
All harvested tissues were demineralized, dehydrated through an ethanol series and embedded in paraffin wax. Sections (thickness, 5μm) were cut, stained with hematoxylin and eosin (Table 2).

Statistics
Data are expressed as mean ± S.E. Statistical evaluation was performed by one-way ANOVA using SAS system.

Results
Fig.1 Representative posteroanterior radiographs and coronal view of CT scans were shown (8 weeks after surgery). Obvious osseous union was observed in group C, compared with the other groups.

Fig.2 In the group C, BMD had increased significantly at 8 weeks after surgery. In the other group, the maximum value was detected at 4 weeks after surgery and decreased afterward slightly.

Table 1. By manual palpation, fusion was detected all the specimens in the group C.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal No.</th>
<th>Score</th>
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<tbody>
<tr>
<td>A</td>
<td>1 2 3</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>4 5 6</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>7 8 9</td>
<td>+</td>
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Notes)  +: solid (sufficient union)  -: not solid (insufficient union)

Table 2. Histological findings were shown. In the group C, the junctions between the implant and host bone were bony fused completely. In the group A, bony union was not observed but resorption of the autograft appeared.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tbody>
<tr>
<td>Left</td>
<td>1 2 3</td>
<td>4 5 6</td>
<td>7 8 9</td>
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<tr>
<td>Right</td>
<td></td>
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<tr>
<td>Degeneration and/or resorption of implant</td>
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<tr>
<td>Bony union between implant and host bone</td>
<td>2+ 2+ 2+</td>
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<tr>
<td>Bony fusion</td>
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Notes)  -: No change  -: Very slight  +: Slight  2+: Moderate  3+: Marked

Discussion
This study demonstrated that BMP-7/IP-CHA composite can induce sufficient bone formation in pores and around the implant, leading to intertransverse process fusion in the canine model. Thus, the composite can be an excellent alternative to autograft and/or calcium phosphate ceramics bone substitute in posterolateral lumbar spinal fusion treatment.

In addition, this dose of BMP-7 (1mg / side) is much smaller than that in previous report (3.5mg / side; using type I bovine collagen as a carrier). This finding suggests that BMP-7/IP-CHA composite potentiates the activity of BMP-7 in bone regeneration, and may reduce the required amount of BMP-7.