CLINICAL ARTICLE

Effectiveness and Feasibility of Injectable *Escherichia coli*-Derived Recombinant Human Bone Morphogenetic Protein-2 for Anterior Lumbar Interbody Fusion at the Lumbosacral Junction in Adult Spinal Deformity Surgery: A Clinical Pilot Study

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Abstract

Objective: To explore the effectiveness and feasibility of injectable *Escherichia coli*-derived recombinant human bone morphogenetic protein-2 (injectable E-rhBMP-2, a combination of *E. coli*-derived recombinant human bone morphogenic protein-2 and a hydrogel type beta-tricalcium phosphate carrier) as a bone substitute for anterior lumbar interbody fusion (ALIF) of the lumbosacral junction in adult spinal deformity (ASD) patients.

Methods: A prospective single-institution therapeutic exploratory trial was conducted. Twenty patients (average age: 69.1 years; 19 female and one male; average fusion level: 7.95) diagnosed with ASD with sagittal imbalance who underwent surgical treatment including ALIF at the lumbosacral junction from December 2017 to January 2019 were evaluated. Injectable E-rhBMP-2 was prepared by dissolving 3 mg of *E. coli*-derived recombinant human bone morphogenetic protein-2 in 1.5 ml H_2O and mixing *in situ* with 9 g hydrogel type beta-tricalcium phosphate. This bone graft substitute was loaded onto a metal ALIF cage and L_5–S_1 ALIF was performed in routine manner. Then posterior column osteotomy with multilevel oblique lumbar interbody fusion or pedicle subtraction osteotomy with accessory rod technique was performed to restore sagittal balance. Patients were followed up for 12 months. CT-based fusion rates were examined at 6 and 12 months after surgery. Also, clinical outcomes (Oswestry Disability Index [ODI], Visual Analog Scale [VAS] score of the back and leg) were evaluated at 6 and 12 months after surgery. All postoperative adverse events were evaluated for the association with injectable E.BMP-2.

Results: Of the 20 patients, loss to follow-up occurred with one patient at 6 months after surgery and one patient at 12 months after surgery, resulting in a total of 18 patients who were available for follow-up. Six months after surgery, 68.4% patients achieved solid fusion. Twelve months after surgery, 100% fusion rate was achieved. Compared to baseline values, ODI scores improved to 45.8% and 63.7%, VAS (back) improved to 69.2% and 72.8%, and VAS (leg) improved to 49.2% and 64.8%, respectively, at 6 and 12 months after surgery ($p < 0.001$ for all). Ten cases of adverse events occurred. But no adverse events were associated with injectable E-rhBMP-2.

Conclusion: Injectable E-rhBMP-2 will be an effective bone graft substitute when achieving solid interbody fusion in the lumbosacral junction.

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Introduction

Achievement of solid fusion of the lumbosacral junction (L_5–S_1 level) is an important factor for long-term prognosis and the prevention of complications with long-segment fusion following deformity correction of adult spinal disease (ASD).[1–4] The lumbosacral junction has a high rate of nonunion due to low bone quality, complex anatomy, and the application of high biomechanical forces following long-segment fusion.[5,6] Thus, a variety of methods, such as autogenous bone graft, anterior column support, and sacropelvic fixation, have been used to increase fusion rates.[3,6,7] Nevertheless, nonunion of the lumbosacral junction is a major challenge in performing long-segment fixation in ASD patients.

The gold standard for fusion of the lumbosacral junction is an autogenous bone graft, but the amount that can be obtained is limited and there is a potential for complications at the donor site. For these reasons, the use of several other bone substitutes, such as local bone, allogenic bone, demineralized bone matrix, and recombinant human bone morphogenetic protein-2 (rhBMP-2), has been preferred as alternatives to autogenous bone grafts. Among these, rhBMP-2 has greater osteoinductivity than demineralized bone matrix, and Chinese hamster ovary cell-derived rhBMP-2 (C-rhBMP-2) has been used in various bone fusion surgeries.[8] This C-rhBMP-2 has been widely used because it is associated with excellent fusion outcomes in spinal fusions for ASD. Many studies evaluated the effect of C-rhBMP-2 and fusion rates were reported above 90%.[9–13] However, this approach is limited by concerns of complications from the animal cell origin, as well as its high cost resulting from low yield.[14,15] To overcome this, *Escherichia coli*-derived rhBMP-2 (E-rhBMP-2) has been developed. E-rhBMP-2 is produced through the inclusion bodies of *E. coli*, eliminating the risk of antibody formation or disease transmission, and this is associated with a yield advantage of up to 99%.[15,16] E-rhBMP-2 was confirmed to have equivalent bone fusion performance to C-rhBMP-2.[17–19] Nonetheless, there are not many reports on the outcomes of interbody fusion in the lumbosacral junction using E-rhBMP-2.

Meanwhile, since rhBMP-2 is rapidly absorbed in the body, a carrier is needed to maintain its osteoinductive function. Absorbable collagen sponge (ACS), hydroxyapatite (HA), or beta-tricalcium phosphate (β-TCP) can be used as carriers. Among them, HA carrier used with E-rhBMP-2 for posterolateral fusion achieved 100% fusion rate within 6 months.[20,21] HA has good osteoconductivity and biocompatibility, but the absorption rate is low and has difficulty in further bone remodeling.[22] In contrast, β-TCP is completely resorbable and has high affinity to rhBMP-2.[23–25] Compared with HA, Lee *et al.* proposed that β-TCP might be a useful carrier of E-rhBMP-2 for new bone formation.[22] By animal study, β-TCP was suggested to be an effective carrier of E-rhBMP-2 for spinal fusion.[15] Recently, Wang *et al.* applied E-rhBMP-2 with β-TCP carrier on anterior cervical discectomy and fusion and reported 100% fusion rates with an improvement of clinical symptoms within 12 months.[26] Using this β-TCP carrier in hydrogel type has the advantage of high osteoconductivity, biocompatibility, and fluidity, which enables the transplantation of grafts onto irregular surfaces.[27] Thus, combining E-rhBMP-2 with a hydrogel type β-TCP carrier and using it in the form of injectable E-rhBMP-2 could help to increase the fusion rate.

Therefore, this study aimed to explore the effectiveness and feasibility of injectable E-rhBMP-2 (a combination of 3 mg of E-rhBMP-2 and a 9g of hydrogel type β-TCP carrier [95% purity, 45–75μm porous circular bead and porosity >68%]) as a bone substitute for the fusion of lumbosacral junctions that have high nonunion rates. For this, a prospective single-institution therapeutic exploratory clinical pilot study involving a 1-year postoperative observation of 20 ASD patients was conducted. Injectable E-rhBMP-2 was applied at the L_5–S_1 level of anterior lumbar interbody fusion (ALIF) in patients who underwent long-segment fusion for ASD diagnosed as lumbar degenerative kyphosis (LDK), and the results including radiologic outcomes, fusion rates, clinical outcomes, and adverse events were evaluated.

Materials and Methods

Study Design

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (i) patients who underwent long-segment fusion[3] to S_1 with ALIF L_5–S_1 as a surgical treatment by a single surgeon at a single institution; (ii) a single etiology of LDK, patients who clearly showed atrophy of back musculature on magnetic resonance imaging as a diagnostic criterion for LDK and clinical signs including walking difficulty with stooping, inability to lift heavy objects to the front, difficulty in climbing slopes, and need for elbow support when working in the kitchen, resulting in hard corns on the extensor surfaces.[28–30]

The exclusion criteria were as follows: (i) patients who had undergone previous surgery at the lumbosacral junction; (ii) patients with immunosuppressive or autoimmune diseases; (iii) history of malignant tumors; (iv) patients with fractures, acute infections, bleeding disorders, active systemic infections, bone formation disorders, or infected surgical sites; (v) patients with serious conditions that the investigator

deems might affect surgery (e.g. heart failure, kidney failure, liver failure, uncontrolled blood pressure, diabetes, blood clotting disorders, etc.).

Patients

This study was a prospective single-institution therapeutic exploratory clinical pilot study involving a 1-year postoperative observation of 20 ASD patients who underwent surgical treatment including ALIF at the L_5–S_1 level from December 2017 to January 2019.

Informed consent and basic patient information were obtained on the screening day. Selection/exclusion criteria and vital signs were also assessed. Patients were followed up regularly for 6 and 12 months after surgery. Plain radiographs and lumbar three-dimensional computed tomography (CT) scans were performed along with an evaluation of clinical outcomes at each visit.

Intervention

Approval of the clinical trial plan for injectable E-rhBMP-2 (NOVOSIS Inject; CG Bio Co. Ltd., Seongnam, Gyeonggi-do, Korea) composed of E-rhBMP-2 (CG Bio Co. Ltd., Seongnam, Gyeonggi-do, Korea) and hydrogel type β-TCP (ExcelOS inject; CG Bio Co. Ltd., Seongnam, Gyeonggi-do, Korea) with a pore size of 45–75 μm was obtained from the Ministry of Food and Drug Safety. The safety and efficacy of those materials were evaluated and approved by Korea Ministry of Health and Welfare. After dissolving 3 mg E-rhBMP-2 in 1.5 ml H_2O, it was mixed *in situ* with 9 g hydrogel-type β-TCP. The final bone graft substitute comprised of E-rhBMP-2 -loaded β-TCP hydrogel was loaded onto a metal ALIF cage and used for ALIF L_5–S_1 in a routine manner (Figure 1). Thereafter, posterior column osteotomy (PCO) with multilevel oblique lumbar interbody fusion (OLIF) was performed to restore sagittal alignment.[31] If multilevel OLIF was not feasible due to previous lumbar spinal fusion, pedicle subtraction osteotomy (PSO) was performed. And with concerns about pseudarthrosis, applied accessory rod technique was performed with PSO.

Radiographic Measurements

Plain lateral 14 × 36-inch full-spine radiographs were obtained with the patients standing in a neutral, unsupported, "fists-on-clavicle" position.[32] All radiographs were evaluated using validated software (Surgimap, Nemaris Inc., New York, NY).[33] We evaluated following spinopelvic parameters; sagittal vertical axis (SVA), thoracic kyphosis (TK), lumbar lordosis (LL), pelvic incidence (PI), pelvic tilt (PT), and sacral slope (SS).

Sagittal Vertical Axis

SVA was defined as the horizontal distance between the posterosuperior corner of the sacrum and the C7 plumb line. Optimal and suboptimal sagittal balances were defined as SVA ≤50 mm and > 50 mm, respectively.[34]

Pelvic Parameters

PI was measured using a standing lateral radiograph of the pelvis, and the angle was defined between a perpendicular line from the sacral plate and a line connecting the midpoint of the sacral plate to the bicoxofemoral axis. SS corresponded to the angle between the sacral plate and horizontal plane, and PT corresponded to the angle between a line connecting the midpoint of the sacral plate to the bicoxofemoral axis and vertical plane.[35]

Sagittal Cobb Angles

Cobb angle is defined as the greatest angle at a particular region of the vertebral column when measured from the superior endplate of a superior vertebra to the inferior endplate of an inferior vertebra.[36] And a sagittal Cobb angle is one measured in the sagittal plane such as on lateral radiographs. Sagittal Cobb angles were measured for TK (T_5–L_2) and LL (T_{12}–S_1).[37,38]

Bone Fusion Measurements

For the evaluation of bone fusion, CT-based fusion rates were examined at 6 and 12 months after surgery. Fusion rates were evaluated according to the 4-point grading scale

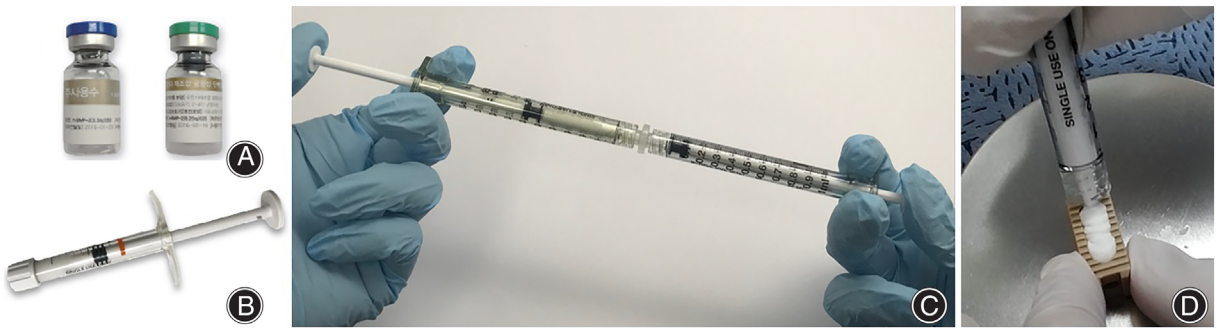

Fig. 1 Injectable *Escherichia coli*-derived recombinant human bone morphogenic protein-2 (E.BMP-2)-loaded beta-tricalcium phosphate (β-TCP) hydrogel. (A) 3 mg E.BMP-2 was dissolved in 1.5 ml H_2O. (B) 9 g hydrogel type β-TCP was used. (C) A and B were mixed *in situ*. (D) The final bone graft substitute (NOVOSIS Inject) was loaded onto an anterior lumbar interbody fusion (ALIF) cage

suggested by Whang *et al.*[39] No evidence of fusion was classified as grade 1, ossification within the disc space but no bridging with the endplate was classified as grade 2, bridging with the end plate less than 50% was classified as grade 3, and bridging more than 50% was classified as grade 4. Grades 3 and 4 were defined as fusion. The evaluation of bone fusion was based on the subjective judgment of an independent radiologist who did not participate in the procedure.

Clinical Outcome Assessments

The percent changes from baseline Oswestry Disability Index (ODI) and Visual Analog Scale (VAS) scores were examined at 6 and 12 months after surgery for the evaluation of clinical symptoms. Percent changes from baseline ODI and VAS scores were calculated as follows: (ODI and VAS scores at each visit − baseline value)/baseline value × 100 (%).

Oswestry Disability Index

ODI is the most commonly used indicator of the condition-specific outcome measure,[40–42] and it consists of 10 items that assess the level of pain and interference with several physical activities; pain intensity, personal care, lifting, walking, sitting, standing, sleeping, sex life, social life, and traveling. Each item asks how the pain affects the activities of daily living and is scored. For each section of six statements the total score is 5. If all 10 sections are completed the score is calculated as follows: total scored out of total possible score ×100. If one section is missed (or not applicable) the score is calculated: (total score/[5 × number of questions answered]) × 100%. The scores are as follows: 0%–20% is considered mild dysfunction, 21%–40% is moderate dysfunction, 41%–60% is severe dysfunction, and 61%–80% is considered as disability. For cases with a score of 81%–100%, the person is either long-term bedridden or exaggerating the impact of pain on their life. The greater outcome percentage, the more extreme the disability.

Visual Analog Scale

VAS is a simple and frequently used method of measuring pain intensity,[43] and the percentage of the pain relief measured by the VAS score is considered a method of the treatment efficacy.[44] The VAS pain scoring standard (scores from 0 to 10) was as follows: 0 = painless; less than 3 = mild pain that the patient could endure; 4–6 = patient was in pain that could be endured and was able to sleep; and 7–10 = patient had intense pain and was unable to tolerate the pain.[45]

Adverse Events Evaluation

To evaluate the safety of injectable E-rhBMP-2, the occurrence and severity of all postoperative adverse events were examined. All unexpected and unintended signs, symptoms, and diseases that occurred to patients during the study were recorded as adverse events. Each event was then evaluated for its association with the injectable E-rhBMP-2.

Statistical Analysis

The Shapiro–Wilk test was used to check for normality in the radiological and clinical results. A paired *t*-test was used for results that showed normal distribution, while the Wilcoxon signed rank test was used for results that failed to satisfy normal distribution. All statistical analyses were performed using SPSS software (version 25.0. IBM Corp., Armonk, NY). A *p*-value of <0.05 was considered statistically significant.

Results

Baseline Characteristics

The mean age at surgery was 69.1 ± 5.5 years. Of the 20 patients, loss to follow-up occurred with one patient at 6 months after surgery and one patient at 12 months after surgery, resulting in a total of 18 patients who were available for follow-up. The upper instrumented vertebra was at T_{10} in 18 cases and at T_9 and at L_2 in one case each. PCO with multilevel OLIF was performed for 16 patients, whereas four

TABLE 1 Baseline characteristics (*N* = 20)

Variables	Value
Age at surgery (years, mean ± SD)	69.1 ± 5.5
Sex	
Female	19
Male	1
Surgical approach	
PCO with multilevel OLIF	16
PSO with accessory rod technique	4
UIV	
T_9	1
T_{10}	18
L_2	1
LIV	
S_1	20
Fused segments (mean ± SD)	7.85 ± 0.90
Spinopelvic fixation	
S_2-alar-iliac screw fixation	20
Smoking	
Current smoker	0
Ex-smoker	4
Non-smoker	16
Drinking	
Current drinker	1
Ex-drinker	9
Non-drinker	10
Comorbidities	
Diabetic mellitus	6
Hypertension	9
Hyperlipidemia	2
Osteopenia	9
Osteoporosis	11
Previous spinal surgery	4
BMD spine (g/cm^2, mean ± SD)	0.907 ± 1.53
BMD femur (g/cm^2, mean ± SD)	0.758 ± 0.14

Abbreviations: BMD, bone mineral density; LIV, lowermost instrumented vertebra; OLIF, oblique lumbar interbody fusion; PCO, posterior column osteotomy; PSO, pedicle subtraction osteotomy; UIV, uppermost instrumented vertebra.

patients underwent PSO. Sacrum 2-alar-iliac (S_2AI) screw fixation was performed on all patients. Eleven patients had osteoporosis and nine patients had osteopenia (Table 1).

Radiographic Outcomes: Spinopelvic Parameters

The average SVA was 187.9 ± 39.6 mm before surgery and −14.5 ± 28.0 mm after surgery, and the average LL was 1.5° ± 16.7° before surgery and −70.9° ± 12.3° after surgery, ($P < 0.001$, for both), resulting in restoration of optimal sagittal balance with appropriate lordosis correction. Significant changes were also observed in the SS, PT, and TK ($P < 0.001$, for all) (Table 2).

TABLE 2 Radiographic outcomes (mean ± SD)

Variables	Preoperative	Postoperative	*t*-value	*P*-value
SVA (mm)	187.9 ± 39.6	−14.5 ± 28.0	20.313	<0.001*
TK (°)	6.8 ± 14.7	29.5 ± 10.6	−9.982	<0.001*
LL (°)	1.5 ± 16.7	−70.9 ± 12.3	21.169	<0.001*
PT (°)	30.2 ± 11.2	5.1 ± 7.7	10.047	<0.001*
SS (°)	24.1 ± 10.2	49.7 ± 8.3	−9.626	<0.001*
PI (°)	54.3 ± 8.5	54.9 ± 8.0	−1.580	0.131

Abbreviations: LL, lumbar lordosis; PI, pelvic incidence; PT, pelvic tilt; SS, sacral slope; SVA sagittal vertical axis; TK, thoracic kyphosis.; *Statistically significant (*P*-value < 0.05).

Fusion Rates

Six months after surgery, 13 of 19 patients (68.4%; disregarding one patient lost to follow-up) achieved solid fusion (Figure 2). Twelve months after surgery, all 18 patients (100%; disregarding two patients lost to follow-up) achieved solid fusion.

Clinical Outcomes

Table 3 lists clinical outcomes of the study group. Patients lost to follow-up were excluded from the analysis of clinical results.

Oswestry Disability Index

The ODI score decreased from 68.5 ± 14.0 before surgery to 36.0 ± 20.8 at 6 months after surgery and 24.4 ± 15.7 at

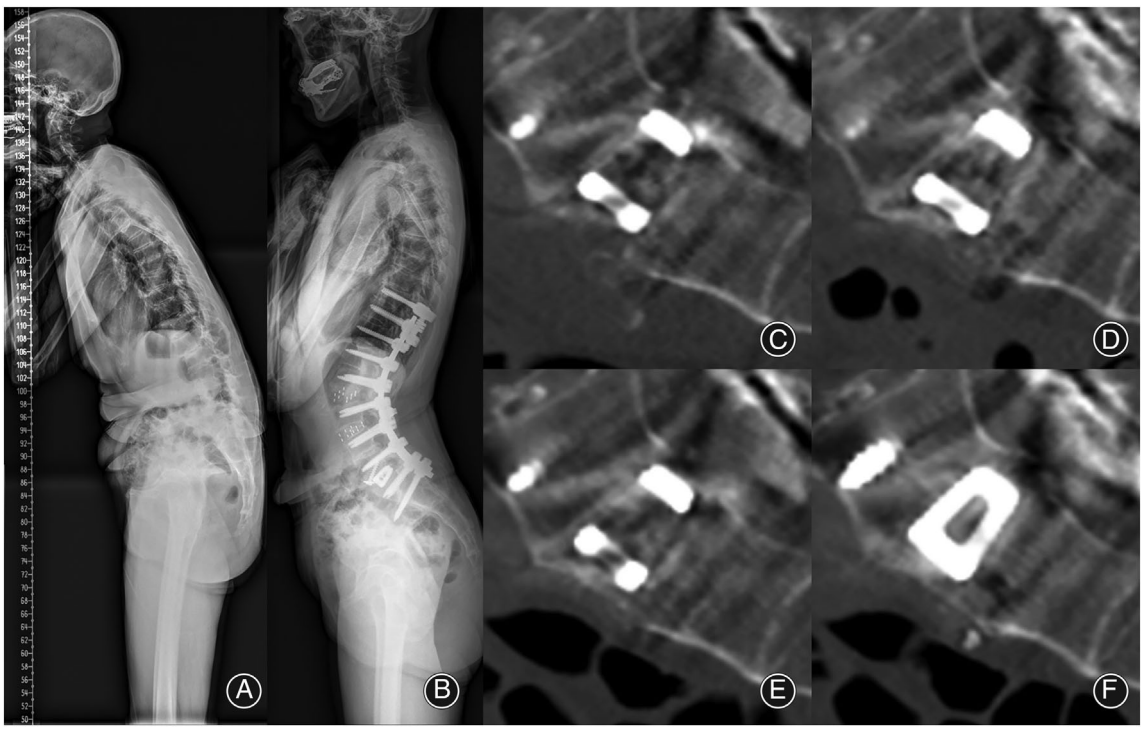

Fig. 2 (A) A 67-year-old women presented to us with degenerative lumbar kyphosis with sagittal imbalance (SVA 166mm, TK 6°, LL −7°, PT 29°, SS 21° and PI 50°). There was bony ankylosis was at L_{4-5}. (B) We performed ALIF on L_5–S_1 with NOVOSIS Inject, OLIF on L_{2-4}, and posterior column osteotomy from T_{10} to S_1 with sacropelvic fixation. Optimal sagittal alignment was maintained until 12 months after surgery (SVA 3 mm, TK 26°, LL −62°, PT 9°, SS 41°). (C) Immediate postoperative state of lumbosacral junction with NOVISIS Inject loaded onto an ALIF cage. (D) Six months after surgery, bridging between the endplates was on progression. (E,F) Twelve months after surgery, grade 4 solid fusion was achieved. Abbreviations: ALIF, anterior lumbar interbody fusion; LL, lumbar lordosis; OLIF, oblique lumbar interbody fusion; PI, pelvic incidence; PT, pelvic tilt; SS, sacral slope; SVA, sagittal vertical axis; TK, thoracic kyphosis

TABLE 3 Clinical outcomes and improvement rates after surgery (mean ± SD)

	Baseline	Postoperative 6 months (improvement rate)	*P*-value*	Postoperative 12 months (improvement rate)	*P*-value*
ODI	68.5 ± 14.0	36.0 ± 20.8 (+45.8%)	<0.001**	24.4 ± 15.7 (+63.7%)	<0.001**
VAS (back)	7.8 ± 2.1	2.1 ± 1.7 (+69.2%)	<0.001**	1.7 ± 1.1 (+72.8%)	<0.001**
VAS (leg)	7.0 ± 1.8	3.4 ± 2.2 (+49.2%)	<0.001**	2.3 ± 1.8 (+64.8%)	<0.001**

Abbreviations: ODI, Oswestry Disability Index; VAS, visual analog scale.; **p*-value was calculated compared with baseline.; ***Statistically significant (*p*-value < 0.01).

12 months after surgery, improving to 45.8% and 63.7%, respectively, compared to baseline values ($P < 0.001$ for all).

Visual Analog Scale

The VAS (back) score decreased from 7.8 ± 2.1 before surgery to 2.1 ± 1.7 at 6 months after surgery and 1.7 ± 1.1 at 12 months after surgery, improving to 69.2% and 72.8%, respectively, compared to baseline values ($P < 0.001$ for all).

The VAS (leg) score decreased from 7.0 ± 1.8 before surgery to 3.4 ± 2.2 at 6 months after surgery and 2.3 ± 1.8 at 12 months after surgery, improving to 49.2% and 64.8%, respectively, compared to baseline values. All scores demonstrated significant improvements compared to baseline values ($P < 0.001$ for all).

Adverse Events

Ten cases of adverse events occurred in five of 20 patients (25.0%; Table 4). No adverse events were associated with injectable E-rhBMP-2. Otherwise, there were no mechanical complications of ASD surgery, such as proximal junctional kyphosis, pseudarthrosis, rod fracture, or hardware failure.

TABLE 4 Complications after surgery

Complications	Patients (%)
Pulmonary edema	3 (15%)
Pulmonary thromboembolism	2 (10%)
Wound infection	1 (5%)
Enterocolitis	1 (5%)
Progressive gait disturbance	1 (5%)
Compression fracture	1 (5%)
Colonic tubular adenoma	1 (5%)

Discussion

This study was a prospective single-institution therapeutic exploratory clinical pilot study to reveal the effectiveness and feasibility of injectable E-rhBMP-2. Although the number of patients were small and follow-up period was short, we achieved 100% fusion rate within 1 year without any adverse effects. Based on these results and more studies conducted in the future, injectable E-rhBMP-2 bone substitute will be widely used to achieve solid fusion in ASD surgery.

In surgical treatment of ASD, C-rhBMP-2 has demonstrated excellent performance as a bone substitute to promote bone fusion. In a previous study involving the use of C-rhBMP-2 for anterior fusion in ASD, Luhmann *et al.* reported a fusion rate of 96% when using 10.8 mg/level of C-rhBMP-2,[10] whereas Mulconrey *et al.* reported a fusion rate of 91% when using 10 mg/level of C-rhBMP-2.[11] Furthermore, Annis *et al.* reported a 97% fusion rate using a relatively low dose (3.2 mg) of C-rhBMP-2 for posterolateral fusion of L_5–S_1 in ASD,[13] similar to what was used in this study. Nonetheless, there is a risk of antibody formation with C-rhBMP-2, as well as a risk of disease transmission.[15] It also has disadvantages of high costs and low yields.[14]

However, E-rhBMP-2 is produced through the inclusion bodies of *E. coli*, eliminating the risk of antibody formation or disease transmission, and this is associated with a yield advantage of up to 99%.[15,16] Cho *et al.* collectively used an E-rhBMP-2 with an HA carrier for posterolateral fusion in spinal stenosis patients, reporting similar bone fusion capabilities as that in an autologous bone.[21] However, to our knowledge, no study has used E-rhBMP-2 for interbody fusion at the lumbosacral junction in ASD patients. Therefore, we combined E-rhBMP-2 and hydrogel type β-TCP (NOVOSIS Inject, a combination of E-rhBMP-2 and a hydrogel type β-TCP carrier) and applied it to L_5–S_1 level ALIF in patients who underwent long-segment fixation for a single etiology of LDK among cases of ASD. We also evaluated the effectiveness and feasibility of this material.

Achievement of 100% Bone Fusion with Injectable E-rhBMP-2

Using an injectable E-rhBMP-2, we achieved 100% fusion rates 12 months after surgery. Although the speed of fusion was slower than that demonstrated in the study by Cho *et al.*[21] where 100% fusion rate was achieved in 6 months, our study showed satisfactory results with a 100% fusion rate at 12 months after surgery. Whereas Cho *et al.* performed single-level posterolateral fusion, our study performed long-segment fixation with an average of 7.9 segments, which imposes a much greater biomechanical stress on the

lumbosacral junction, and this might have slowed down bone fusion. In addition, the sacrum has low bone quality and a complex anatomy, making it vulnerable to nonunion.[5,6] In this study, a 100% fusion rate was obtained using a relatively low dose of 3 mg/level of E-rhBMP-2 in the L_5–S_1 level ALIF compared to that in previous studies using C-rhBMP-2.[10,11]

Hydrogel Type β-TCP as a Carrier for Injectable E-rhBMP-2

Meanwhile, rhBMP-2 needs a carrier to maintain its osteoinductive function because rhBMP-2 is rapidly absorbed in the body. In several studies, ACS, HA, or β-TCP are candidates for the carrier or rhBMP-2. ACS in combination with C-rhBMP-2 gained approval from FDA and is widely used for spinal surgery. But because ACS has low osteoconductivity and low affinity with rhBMP-2, this combination requires lots of ACS.[46] HA has good osteoconductivity and biocompatibility, but after fusion, it has difficulty in further bone remodeling because of low absorption rate.[46] In contrast, β-TCP is completely resorbable with high osteoconductivity and biocompatibility.[24,25] Also, β-TCP has high affinity to rhBMP-2 as a mechanical support of rhBMP-2.[22] Lee *et al.* evaluated HA, β-TCP, and HA/TCP as the carrier for E-rhBMP-2, and they found that E-rhBMP-2 with β-TCP carrier can function as effectively as C-rhBMP-2 with ACS carrier.[22] Using this β-TCP carrier in hydrogel type can increase osteoconductivity, biocompatibility, and fluidity, which enables the transplantation of grafts onto irregular surfaces.[26] Therefore, to increase the efficiency of bone substitute, we applied a hydrogel type of β-TCP in combination with E-rhBMP-2. This hydrogel type of β-TCP is composed of thermosensitive polyethylene oxide and polypropylene oxide block copolymer. It is in a sol state at room temperature and changes into a viscous hydrogel *via in situ* mixing with E-rhBMP-2, which enables easy implantation of the graft inside the cage. Upon implantation, the polymeric hydrogel components are gradually biodegraded or discharged.[47] By using such a moldable and injectable β-TCP hydrogel with E-rhBMP-2, the graft was able to fill the irregular cage surface, and its efficiency was increased as the amount of carrier lost during implantation was minimized. Thus, it can be presumed that the injectable nature of the bone graft substitute helped to promote bone fusion, enabling an excellent fusion rate, while using a relatively small amount of E-rhBMP-2.

Improvement of Clinical Outcomes with Injectable E-rhBMP-2

Along with rapid bone fusion, significant improvements in clinical outcomes were also observed in this study. Many studies have previously demonstrated significant improvements in clinical outcomes with the progression of intervertebral fusion when E-rhBMP-2 was used in ALIF.[9,48–50] Burkus *et al.* demonstrated a dramatic improvement in the ODI score when using E-rhBMP-2 of mammalian cell origin in ALIF, from an ODI score of 52.4 before surgery to 21.4 at 6 months after surgery, and 20.8 at 12 months after surgery.[9] Although direct comparison is difficult because the disease entity was different and long-segment fixation was performed in our research, this study showed a similar significant improvement in clinical outcomes after surgery. However, as this was a single-group study, a comparative analysis with a control group will be needed in the future.

Related Adverse Events with Injectable E-rhBMP-2

We also confirmed the feasibility of injectable E-rhBMP-2. In this study, 10 adverse effects occurred in five patients (25.0%), but there were no complications directly associated with E-rhBMP-2. Enterocolitis and colonic tubular adenoma occurred during follow-up, but these were determined to have no relationship with injectable E-rhBMP-2. Also, we experienced three pulmonary edema and two pulmonary thromboembolisms. Three pulmonary edema cases occurred due to input–output imbalance during operation. They were all treated completely within 3 days with keeping negative input–output balance. Two pulmonary thromboembolism cases occurred immediately after postoperative period. We think that they occurred due to patients' immobile condition because absolute bed rest was performed approximately 3~5 days. But the embolies were very small and patients were treated completely with thrombolytic agents.

There are some known complications directly associated with C-rhBMP-2 use in spinal surgery, such as neuritis, ectopic bone formation, painful seroma formation, vertebral osteolysis, pseudarthrosis, wound infections, and deep vein thrombosis.[51–53] The incidence of such complications has been reported to increase with the dose of C-rhBMP-2.[54] In particular, Carragee *et al.* reported that the incidence of retrograde ejaculation was higher in a C-rhBMP-2 group (8%) than in a control group (1.4%) when C-rhBMP-2 was used in ALIF.[55] However, it was impossible to examine the incidence of retrograde ejaculation in this study as there was only one male patient. Considering improvements in clinical symptoms, there seemed to be no incidence of neuritis or painful seroma. Although there was one case of wound infection, it was due to fat necrosis, which was successfully treated using superficial wound irrigation and drainage and healed without deep infection. It is presumed that no adverse effects associated with E-rhBMP-2 occurred due to the relatively low dosage of E-rhBMP-2. However, more studies are needed to confirm the safety of this material.

Limitations

This study had the following limitations. First, the number of patients was small with a short follow-up period. And this study lacked a power analysis. Larger number of patients with power analysis is needed. With a larger number of patients and a longer follow-up period, there might have been nonunion cases with lower fusion rates, or there could have been an increase in complications. In particular, tracking the incidence of retrograde ejaculation following ALIF with a greater number of male patients is needed. Furthermore, as this study was a single group study, it was impossible to perform a comparative analysis of fusion rates with

another group using autologous iliac bone grafts or a group using C-rhBMP-2. Therefore, evaluation of the outcomes of injectable E-rhBMP-2 through a long-term comparative follow-up study using control groups is needed in the future. Despite such limitations, there were advantages to this study, as it was performed by a single surgeon in the same way with the same etiology of LDK among ASD cases. This study is the first to analyze the effectiveness and feasibility of injectable E-rhBMP-2. The results demonstrated excellent fusion rates without any adverse events in a short 12-month period in the lumbosacral junction, where the risk of nonunion is high. Also, compared with other studies using C-rhBMP-2 for interbody fusion in ASD,[10,11] we achieved satisfactory results with relatively small amount of E-rhBMP-2 (3 mg).

Conclusions

Injectable E-rhBMP-2 (NOVOSIS Inject, a combination of E-rhBMP-2 and a hydrogel type β-TCP carrier) will be an effective bone graft substitute when achieving solid interbody fusion in the lumbosacral junction.

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Conflict of Interest

The authors declare no conflicts of interest.

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