CLINICAL TRIALS

Efficacy of rhBMP-2/Hydroxyapatite on Sinus Floor Augmentation: A Multicenter, Randomized Controlled Clinical Trial

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Abstract: *The aim of this randomized single-blinded active-controlled clinical study was to evaluate the early efficacy of low-dose* Escherichia coli*–derived recombinant human bone morphogenetic protein 2 (rhBMP-2) soaked with hydroxyapatite granules (BMP-2/H) as compared with an inorganic bovine bone xenograft (ABX) in maxillary sinus floor augmentation. In a total of 127 subjects who were enrolled at 6 centers, maxillary sinus floors were augmented with 1 mg/mL of rhBMP-2 (0.5 to 2.0 mg per sinus) and BMP-2/H (0.5 to 2.0 g;* n = 65*) or with ABX alone (0.5 to 2.0 g;* n = 62*). Core biopsies were obtained 3 mo after the augmentation surgery and were analyzed histomorphometrically. The mean new bone formation with BMP-2/H and ABX augmentation was 16.10% ± 10.52% and 8.25% ± 9.47%, respectively. The BMP-2/H group was noninferior to the ABX group; the lower limit of the 1-sided 97.5% confidence interval for the difference between the 2 groups was calculated as 4.33%, which was greater than the prespecified noninferiority margin of –3.75%. An additional test with the Wilcoxon rank-sum test with a 2-sided 5% significance level showed that bone formation between the 2 groups was significantly different (*$P < 0.0001$*). The soft tissue and residual graft areas showed no significant differences between the groups. With regard to safety, no significant difference between the 2 groups was observed; there was no significant increase in the amount of rhBMP-2 antibody in the serum after BMP-2/H grafting. Our study suggested that low-dose* Escherichia coli*–derived rhBMP-2 with hydroxyapatite was effective in early stages for enhanced bone formation after maxillary sinus floor augmentation without harmful adverse events (Clinicaltrials.gov NCT01634308).*

Key Words: *Escherichia coli*, bone morphogenetic protein 2, biopsy, clinical efficacy, osteogenesis, comparative study.

Introduction

Bone morphogenetic protein (BMP) is the most powerful osteoinductive growth factor and can trigger the differentiation of mesenchymal stem cells to osteogenic cells for accelerated new bone (NB) formation (Jung et al. 2003; Triplett et al. 2009; Carragee et al. 2011). Recombinant human bone morphogenetic protein 2 (rhBMP-2) can be produced in large quantities using gene amplification in mammalian Chinese hamster ovary (CHO) cells or prokaryotic microorganisms, such as *Escherichia coli* (Carreira et al. 2014). While native mature BMP-2 and CHO cell–derived rhBMP-2 (CHOrhBMP-2) are a form of glycosylated homodimer (Wozney et al. 1988; Celeste et al. 1990), *Escherichia coli*–derived rhBMP-2 (ECrhBMP-2) has a different structure from the native form because of a lack of glycosylation in the prokaryotic system (Long et al. 2006). Nevertheless, it has been reported that unglycosylated ECrhBMP-2 has a similar osteoinductive effect

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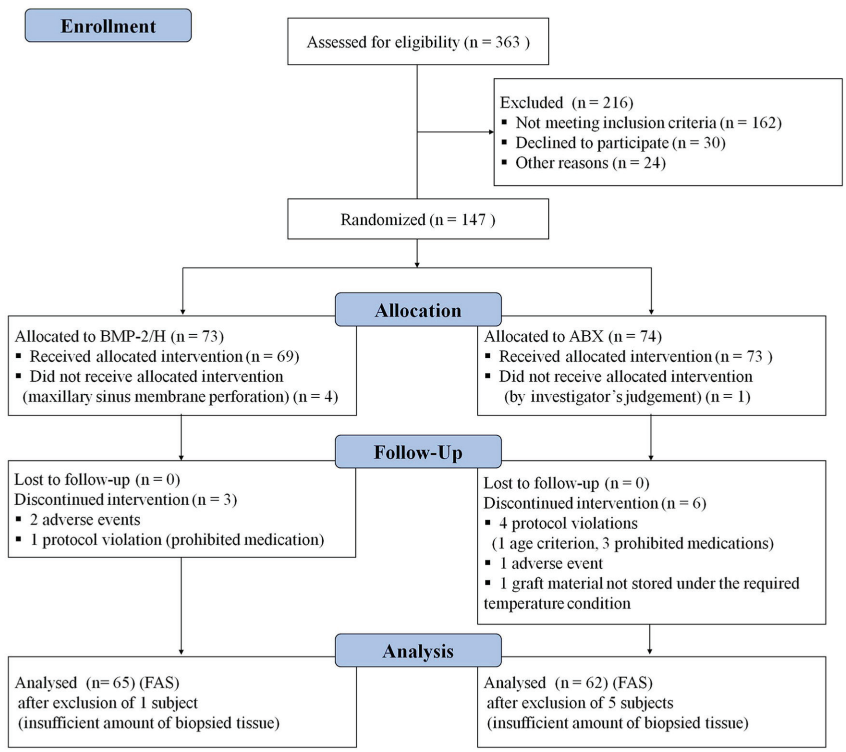


Figure 1.
Flow diagram of a multicenter randomized clinical trial of low-dose *Escherichia coli*–derived recombinant human bone morphogenetic protein 2 with hydroxyapatite granules (BMP-2/H) for maxillary sinus floor augmentation as compared with inorganic bovine bone xenograft (ABX). FAS, full analysis set.

with CHOrhBMP-2 in both in vivo and ex vivo studies (Bessho et al. 2000; Yano et al. 2009; Kim et al. 2011). CHOrhBMP-2 has been approved by the Food and Drug Administration and is commercially available. However, large-scale production from mammalian cells can be challenging and is associated with high costs, while large-scale production of ECrhBMP-2 is relatively easy and has lower associated costs. Purified ECrhBMP-2 can be problematic because of the intense aggregation of the product; however, the product can be made soluble by advances in protein purification technology while maintaining osteoinductive activity using ex vivo biochemical processing (Yano et al. 2009; Zhang et al. 2010). Although a few studies have reported comparable bone regeneration efficacy between ECrhBMP-2 and CHOrhBMP-2 in in vivo and ex vivo experiments (Wozney et al. 1988; Bessho et al. 2000; Kim et al. 2011), CHOrhBMP-2 has been widely used in clinical applications (Boyne et al. 2005; Herford et al. 2007; Triplett et al. 2009; de Freitas et al. 2013; Neovius et al. 2013) because it is commercially available and has been approved by the Food and Drug Administration.

Maxillary sinus floor augmentation (MSFA) with a bone graft is a general procedure for implant placement in the maxillary molar area with an alveolar height deficiency (Boyne and James 1980; Peng et al. 2013). The amount of bone graft for MSFA can be controlled by the vertical and horizontal extension of sinus elevation during surgery. In MSFA, it is possible to obtain a small biopsy of regenerated bone material via the transalveolar approach (Lee et al. 2006; Rickert et al. 2011) or above the upper end of dental fixtures through the lateral sinus wall approach (Bassil et al. 2013; Silvestri et al. 2013; Xavier et al. 2015) without damaging the implant stability or prognosis. The first 2 randomized controlled clinical trials combined with an absorbable collagen sponge (ACS) carrier in the dental field showed that the induced bone in the rhBMP-2/ACS group was significantly denser than that in the bone graft group. No marked differences were found in the histologic parameters between the 2 groups, and no adverse events were deemed related to the rhBMP-2/ACS treatment (Boyne et al. 2005; Triplett et al. 2009).

To date, there is no clinical report regarding the bone-forming effect of ECrhBMP-2 in a randomized multicenter study on MSFA with a large number of patients. The hypothesis in this clinical trial was that NB formation by ECrhBMP-2 delivered with hydroxyapatite granules (BMP-2/H) would be noninferior to NB formation by an inorganic bovine bone xenograft (ABX) in the early stages after MSFA. This study also evaluated its safety.

Materials and Methods

Clinical Trial Design

This randomized active-controlled clinical trial was conducted in 6 university hospitals. It was a prospective parallel study with a single-blind design for subjects (Fig. 1). The 5-step study sequence (5 visits per subject) and the contents are detailed in the Appendix.

Graft Materials and Subjects

Graft materials were BMP-2/H (Novosis-Dent) and ABX (Bio-Oss) for the control. The amount of graft materials that were implanted was determined by the subject's individual sinus size, and a void space was surgically created during MSFA depending on the planned number of dental implants. The total amount of graft materials that were transplanted for 1 MSFA was between 1.0 g and 2.0 g in most cases; 0.5 g was rarely applied for a small sinus void volume. Corresponding to the amount of graft materials, the total dose of ECrhBMP-2 was between 0.5 mg and 2.0 mg. Graft material information was detailed in the Appendix.

The target number of subjects in each group (BMP-2/H and ABX) was 62. The method describing how the sample size was calculated is documented in the Appendix. Factoring in a dropout rate of 15% (n = 11), we determined that 73 people should be part of each group and should be included in this study. Among the 363 candidates who participated in the screening, 216 were excluded, and 147 were enrolled according to the inclusion and exclusion criteria. After randomization, 73 subjects were assigned to the BMP-2/H group and 74 to the ABX group (Fig. 2). The inclusion and exclusion criteria are described in the Appendix.

Surgical Procedure and Radiologic Evaluation of MSFA

Prior to the study, principal investigators at the participating institutions established a consensus for a standardized surgical procedure and postoperative medications. During the patient's second visit, surgery was performed under local infiltration anesthesia with 2% lidocaine. The detailed surgical procedure and radiologic evaluation are described in the Appendix.

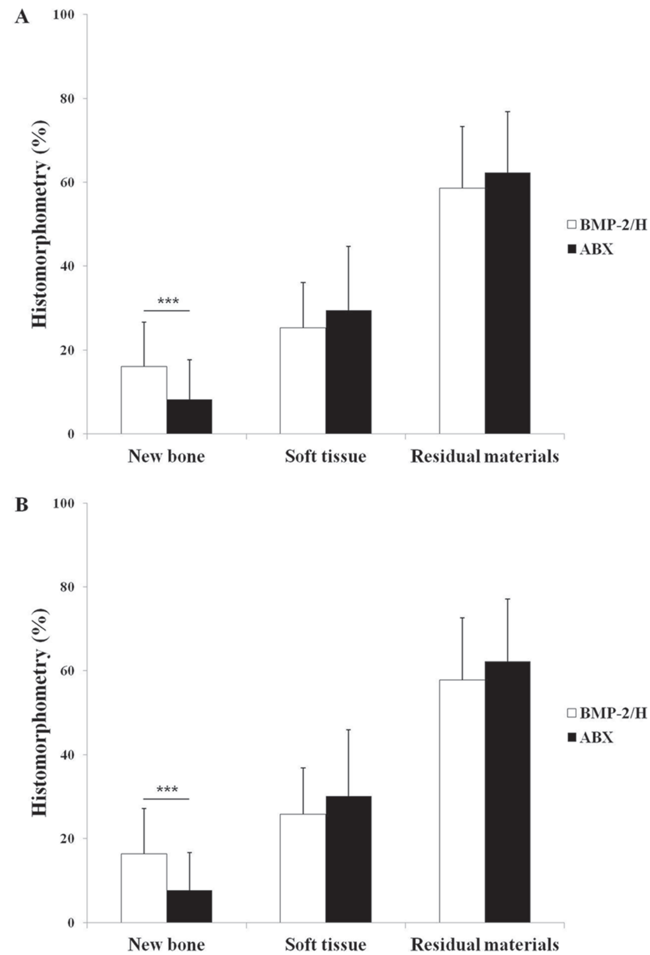
Biopsy

During the fourth visit, the mucoperiosteal flap was raised to the upper margin of the bony window that was formed during the second visit, and 1 to 3 dental implants were placed at the premolar or molar area according to the conventional procedure. Thereafter, a biopsy was taken above the upper end of the implant with a trephine bur that was sized with a 5-mm inner diameter via the lateral window made in the previous MSFA surgery (Appendix Fig. 2). The trephine bur containing biopsied tissue was put into a vessel with 10% formalin solution for fixation. The wound was carefully closed.

Histomorphometric Efficacy Analysis

The procedure for the preparation of histologic staining is described in the Appendix. The histomorphometric analysis was performed in terms of the total area of the biopsied tissue and consisted of the evaluation of the area with 1) newly formed bone (primary outcome), 2) soft tissue including bone marrow, and 3) residual graft materials (secondary outcome) in relation to the total area, expressed as percentages. Two maxillofacial surgeons who did not participate in this study independently analyzed the histologic specimens using Image J (National Institutes of Health, Bethesda, MD, USA). There were no changes to trial outcome measurements after the trial commenced.

Figure 2.
Bone morphogenetic protein 2 with hydroxyapatite granules (BMP-2/H) and inorganic bovine bone xenograft (ABX) efficiency using histomorphometric analysis in the full analysis set (**A**) and the per-protocol set (**B**).



Safety

Safety is described in the Appendix.

Statistical Analysis

The data obtained from the subjects in this clinical study were classified into the full analysis set (FAS), the per-protocol (PP) set (grade II), and the safety set. The main population of this study was the FAS, and additional analysis was performed in the PP set; safety data were analyzed in the safety set. Noninferiority of the primary outcome (NB) was tested by obtaining a 1-sided 97.5% confidence interval for the difference of mean NB (%) between the groups and then by comparing the lower limit of this confidence interval to the predetermined noninferiority margin of –3.75%. The comparisons of other differences were performed by 2-sided tests at a 5% significance level. The differences of the soft area (%) and residual graft materials area (%) between the groups were

Table 1.
Histomorphometric Comparison between Recombinant Human BMP-2/H and ABX in the Full Analysis Set (n = 127; in Percentages)

	BMP-2/H (n = 65)	ABX (n = 62)
New bone[a]		
Mean ± SD	16.10 ± 10.52[b]	8.25 ± 9.47[b]
Median	14.75	4.34
Max, min	42.30, 0.00	30.00, 0.00
Soft tissue		
Mean ± SD	25.27 ± 10.86	29.44 ± 15.31
Median	24.65	25.96
Max, min	57.49, 0.00	75.75, 0.00
Residual materials		
Mean ± SD	58.64 ± 14.61	62.31 ± 14.57
Median	58.72	60.90
Max, min	98.12, 22.58	100.00, 24.25

ABX, inorganic bovine bone xenograft; BMP-2/H, bone morphogenetic protein 2 with hydroxyapatite granules; NS: not significant.
[a]Noninferiority test: lower limit of 1-sided 97.5% confidence interval, 4.33; noninferiority margin, –3.75.
[b]Superiority test: $P < 0.0001$; otherwise, P value for each variable: not significant. Statistics of new bone, soft tissue: Wilcoxon rank-sum test. Statistics of residual materials: independent t test.

tested with the independent t test or the Wilcoxon rank-sum test depending on the data characteristics. The detailed methods for the statistical evaluation of safety are described in the Appendix.

Results

Subjects

This randomized clinical trial ended when it reached its sample size goal and its length of follow-up goal. A total of 147 subjects (73 for BMP-2/H, 74 for ABX) were randomized; of these, a total of 133 subjects (90.48%; 66 for BMP-2/H, 67 for ABX) completed the study, and 14 subjects (9.52%; 7 for BMP-2/H, 7 for ABX) withdrew from the study because of sinus membrane perforation during surgery, protocol violation, or adverse event occurrence. Among the 147 subjects who were assigned to the study, 142 received graft materials for MSFA (Safety set.). Of the 133 subjects who completed the study, 127 were classified to the FAS (65 for BMP-2/H, 62 for ABX) after removal of 6 subjects in whom the amount of biopsied tissue was insufficient to evaluate the efficacy (1 for BMP-2/H, 5 for ABX). A total of 116 subjects belonged to the PP set (60 for BMP-2/H, 56 for ABX) because of the additional exclusion of subjects with protocol violations (5 for BMP-2/H, 6 for ABX; Fig. 1).

In the main population (FAS), subjects in the BMP-2/H group were 53.91 ± 6.81 y old, and the ratio between male and female subjects was 46:19 (70.77%:29.23%). Subjects in the ABX group were 53.15 ± 6.77 y old, and the ratio between male and female subjects was 47:15 (75.81%:24.19%). Six subjects were fertile women who had negative pregnancy test results; all other women were infertile or postmenopausal. There were no statistically significant differences between the groups in sex, age, height, body weight, or smoking status. The relevant data are presented in Appendix Table 1.

Efficacy

The main study population was the FAS group. NB in the biopsies of BMP-2/H and ABX were 16.10% ± 10.52% and 8.25% ± 9.47%, respectively, with a difference of 7.85% between the 2 groups. The lower limit of the 1-sided 97.5% confidence interval for the difference between 2 groups was calculated as 4.33%, which was greater than the prespecified noninferiority margin of –3.75%; therefore, the BMP-2/H group was noninferior to the ABX group. In the PP set, the difference of NB between the 2 groups was 8.74%, and the lower limit of the 1-sided 97.5% confidence interval was 5.09%, which was greater than the prespecified noninferiority margin. Therefore, as with the FAS, the BMP-2/H group was noninferior to the ABX group. The augmentation volumes that were evaluated in computed tomography in the test and control groups were 5.23 ± 0.34 mm^3 and 5.73 ± 0.25 mm^3, respectively; augmentation volumes were not significantly different.

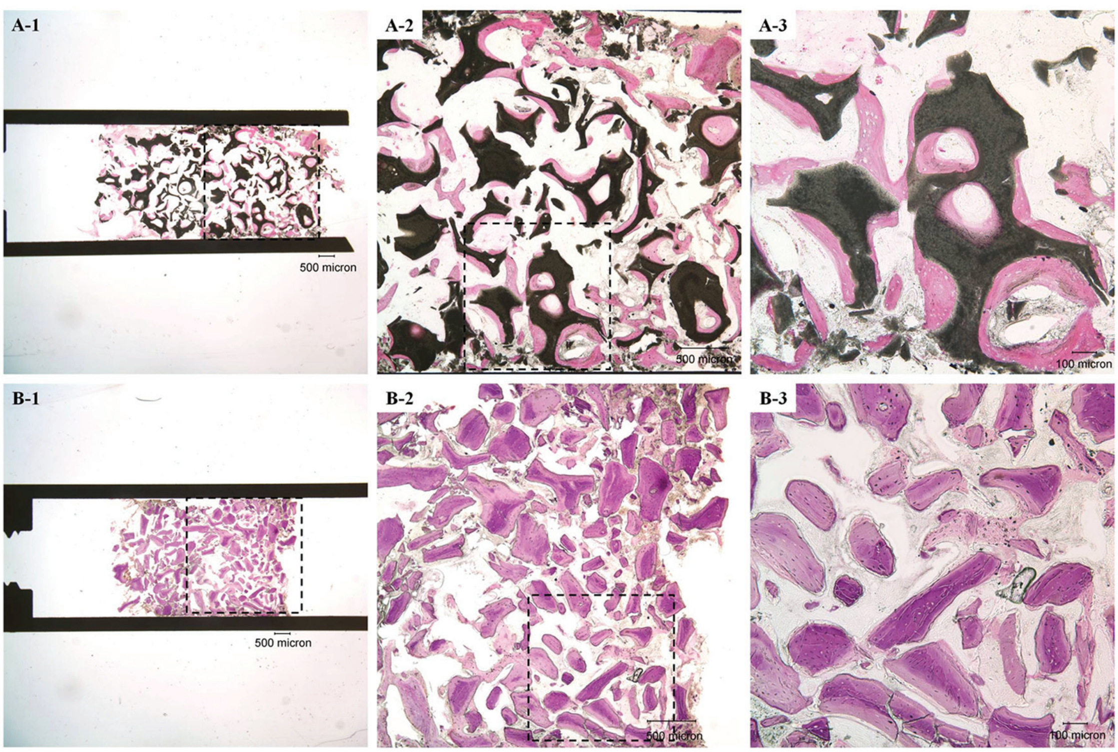
The lower limit of a 2-sided 95% confidence interval for the difference between the 2 groups was 4.33% and did not include zero; the additional superiority test by the Wilcoxon rank-sum test with a 2-sided 5% significance level showed that NB between the 2 groups was significantly different ($P <$ 0.0001; Table 1, Fig. 2A). Therefore, BMP-2/H was shown to be more effective (minimum, 4.33%; maximum, 11.37%) than ABX, even though no hypothesis for the BMP-2/H group being superior to the ABX group was established. The histomorphometric values of NB formation, soft tissue, and residual graft materials are presented in Table 1. This tendency was similar in the PP group; NB formation was also significantly higher in the BMP-2/H group (16.39% ± 10.73%) than in the ABX group (7.64% ± 9.00%; $P <$ 0.0001; Appendix Table 2, Fig. 2B). Comparison of soft tissue and residual graft material areas in the FAS and PP set revealed no significant difference between BMP-2/H (25.27% ± 10.86% and 25.75% ± 11.08%, respectively) and ABX (29.44% ± 15.31% and 30.09% ± 15.89%, respectively; Table 1 and Appendix Table 2; Fig. 2A, B). There were no differences in the histomorphometric values between male and female subjects in the FAS (Appendix Tables 3, 4) or among age groups (40s, 50s, and 60s) for BMP-2/H and ABX (Table 2, Appendix Table 5). Pearson's correlation coefficient showed

Table 2.
Histomorphometric Comparison between Recombinant Human BMP-2/H and ABX Depending on the Ages in Full Analysis Set (n = 127; in Percentages, Mean ± SD)

	40 to 49 y		50 to 59 y		60 to 69 y	
	BMP-2/H (n = 18)	ABX (n = 20)	BMP-2/H (n = 33)	ABX (n = 31)	BMP-2/H (n = 14)	ABX (n = 11)
New bone	16.91 ± 8.82[a]	9.65 ± 10.76[a]	15.28 ± 11.34[b]	6.36 ± 7.93[b]	17.00 ± 11.08	11.01 ± 10.73
Soft tissue	24.90 ± 10.99	29.70 ± 17.08	24.69 ± 11.56	32.71 ± 14.41	27.09 ± 9.46	19.76 ± 10.80
Residual materials	58.19 ± 13.05	60.64 ± 15.18	60.04 ± 15.94	60.94 ± 13.19	55.91 ± 13.76[a]	69.24 ± 16.47[a]

Statistics of new bone and soft tissue (50 to 59 y): Wilcoxon rank-sum test. Statistics of soft tissue (40 to 49 y, 60 to 69 y), residual materials: independent t test. Unless notated, P value for each variable: not significant.
ABX, inorganic bovine bone xenograft; BMP-2/H, bone morphogenetic protein 2 with hydroxyapatite granules; NS: not significant.
[a]$P < 0.05$.
[b]$P < 0.001$.

Figure 3.
Representative histologic images for bone morphogenetic protein 2 with hydroxyapatite granules and inorganic bovine bone xenograft.



no significant correlation between the age and histomorphometric data in the FAS (Appendix Table 6). Representative histologic images for BMP-2/H and ABX are presented in Figure 3.

Safety

Among adverse events that could be related to BMP-2/H, 8 subjects had prolonged swelling for >2 wk. The swelling subsided within 3 wk in 5 subjects, within 4 wk in 1 subject, and within 5 wk in 2 subjects. However, the swelling did not cause wound dehiscence, because there was no complication with wound healing. ABX did not show any prolonged swelling. Other adverse events and serious adverse events are described in the Appendix.

Laboratory analysis indicated that none of the values showed a significant difference within the group, and there was no significant increase in the amount of ECrhBMP-2 antibody after BMP-2/H grafting (Appendix Table 9).

Discussion

The present study investigated the early efficacy of ECrhBMP-2 soaked in hydroxyapatite in MSFA using histomorphometric analysis of biopsied tissue compared with that of ABX. In previous studies with a randomized study design for MSFA, the efficacy of CHOrhBMP-2 was mainly evaluated by computed tomography in terms of increased height, width, and density of bone tissue in comparison with an autogenous bone graft alone or plus an allograft with a lack of histomorphometric results (Boyne et al. 2005; Triplett et al. 2009). A noninvasive method, if it is adequate, would be preferred to an invasive method, such as a biopsy, to evaluate the efficacy. Nevertheless, histomorphometric analysis is essential for evaluation of new and early bone formation in which the bone density is insufficient for computed tomography evaluation.

The ABX is a bovine hydroxyapatite, and it has a natural porous trabecular architecture and a fine crystalline structure; its interconnecting macro- and micropores promote revascularization and migration and subsequent attachment of osteogenic cells (Piattelli et al. 1999; Bassil et al. 2013). It has frequently been used as a comparator biomaterial with many other bone substitutes in animal or clinical studies (Yildirim et al. 2000; Jung et al. 2003; Lee et al. 2006; Bassil et al. 2013; Silvestri et al. 2013). The hydroxyapatite mixed with rhBMP-2 in our study had similar particle and pore size and similar material architecture to ABX. The hydroxyapatite alone functioned successfully as a carrier for ECrhBMP-2 to improve the fusion rate according to the increase in the rhBMP-2 dose in a spine fusion animal study (Lee et al. 2012); synthetic calcium-phosphate cement was also determined to be an efficient carrier for rhBMP-2 in the critical-size supra-alveolar peri-implant defect of canine mandibles (Wikesjo et al. 2002). ABX or alloplastic bone substitutes such as hydroxyapatite are most frequently used for alveolar bone or MSFA to avoid disadvantageous aspects of autogenous bone grafts, such as donor site morbidity and unpredictable resorption rate (de Lange et al. 2014; Schmitt et al. 2015). As carriers of rhBMP-2, those bone substitutes can maintain the graft volume during wound closure and provide mechanical strength until woven bone is induced by rhBMP-2 and changed to lamellar bone. In contrast, ACS might not maintain porous structural integrity by covering soft tissue and external force during bone regeneration; ACS has been shown to be less effective in an animal study (Lu et al. 2013). In the report from Kao et al. (2012), however, the addition of rhBMP-2/ACS to ABX had a negative effect on bone formation after MSFA, as compared with ABX alone.

Two previous clinical trials reported that rhBMP-2/ACS safely induced adequate bone growth for placement of endosseous dental implants 6 to 12 mo after MSFA (Boyne et al. 2005; Triplett et al. 2009). In the present study, the amount of NB formation 3 mo after MSFA was measured to evaluate whether rhBMP-2 could enhance osteogenesis to such an extent that the interval between MSFA and implant placement could be reduced to 50% of the conventional 6-mo waiting period. The bone biopsy showed that the amount of NB formation was 16.10% ± 10.52% in the BMP-2/H group, which was comparable with ABX alone after MSFA in the literature; NB formation ranged from 14.7% ± 5.0% at 6.8 mo (Yildirim et al. 2000), 17.6 ± 2.8% at 8 mo (Bassil et al., 2013) and 18.3 ± 5.4% at 6 mo (Lee et al. 2006). Given that dental implants are usually placed 6 mo after MSFA using ABX, our results suggest that dental implants might be inserted 3 mo after MSFA using BMP-2/H. However, further clinical studies are necessary for the evaluation of implant stability or long-term success rate.

The other concern regarding rhBMP-2 therapy is its effective dose for bone regeneration. A high dose of rhBMP-2 can cause undesirable complications, such as extensive swelling, seroma formation, cystic bone lesion, and cancer development (Leknes et al. 2008; Wikesjo et al. 2008; Carragee et al. 2013). An adequate dose will vary depending on the carrier, with or without sustained releasing properties, and on the individual response of stem cells or progenitor cells to rhBMP-2 because different persons may respond differently to the same dose. Although many animal experiments have been conducted to evaluate the effective dose and carrier for rhBMP-2 (Nam et al. 2009; Han et al. 2010; Kim et al. 2011), an adequate dose for humans, especially for human jaw procedures, has not been established. In the study by Boyne et al. (2005), 1.5 mg/mL of rhBMP-2 was more effective than 0.75 mg/mL for MSFA, where the mean total dose of rhBMP-2 that was implanted per sinus was 8.9 mg (range, 5.2 to 12.0 mg) for subjects with 0.75 mg/mL and 20.8 mg (range, 10.8 to 24.0 mg) for subjects with 1.50 mg/mL. In another study with a dose of 1.5 mg/mL of rhBMP-2 (Triplett et al. 2009), the mean total dose that was implanted per sinus was 19.4 mg (range, 1.6 to 24.0 mg). This dose is very high versus the total dose (0.18 ± 0.13 mg) used for lateral alveolar augmentation in the study by Jung et al. (2003) or the total dose applied in the present study (0.5 to 2.0 mg per sinus). Further prospective clinical studies with different rhBMP-2 doses and delivery methods should be conducted not only to find effective doses for clinical applications but also to reduce undesirable complications and costs from rhBMP-2.

Most adverse events in the present study were related to surgical procedures and not to the graft materials; there was no significant difference between the 2 graft materials, and this result is consistent with other studies (Boyne et al. 2005; Triplett et al. 2009). The pronounced adverse event that was associated with rhBMP-2 was prolonged facial swelling, similar to other studies (Boyne et al. 2005; Triplett et al. 2009). Prolonged swelling subsided within 5 wk without any further complications, and there was no significant increase in the rhBMP-2 antibody after ECBMP-2/H grafting. In other clinical trials, where the total dose per subject ranged from 6 to 48 mg (Boyne et al. 2005) or from 6.4 to 48.0 mg (mean, 28.3; Triplett et al. 2009), 2 of 35 subjects (Boyne et al. 2005) and 2 of 78 subjects (Triplett et al. 2009) developed treatment-emergent elevation in anti-rhBMP-2 antibodies. In those studies, the immunogenic response to rhBMP-2 was transient, and no clinical manifestations of an immune response or a neutralizing effect of the biological activity of rhBMP-2 were identified in any of the subjects.

In conclusion, this clinical study showed that NB formation by ECrhBMP-2 delivered with hydroxyapatite granules was noninferior to NB formation by an inorganic bovine bone xenograft in the early stages after MSFA. An additional test with the Wilcoxon rank-sum test showed that the former had significantly higher NB formation than the latter. Side effects were minimal, and prolonged facial swelling was the pronounced adverse effect.

Author Contributions

H.J. Kim, J.H. Chung, S.Y. Shin, contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript; S.I. Shin, S.B. Kye, N.K. Kim, T.G. Kwon, J.Y. Paeng, J.W. Kim, O.H. Oh, M.S. Kook, H.J. Yang, contributed to data acquisition and analysis, critically revised the manuscript; S.J. Hwang, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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