Effect of recombinant human bone morphogenetic protein-2 on mandibular distraction osteogenesis

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ABSTRACT Objective. This study aimed to evaluate the effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on mandibular distraction at routine and rapid distraction rates. Methods. Eighteen New Zealand white rabbits were randomly assigned to two groups, one treated at a routine distraction rate (0.9 mm/day) and the other at a rapid distraction rate (2.7 mm/day). The rhBMP-2 was injected into one side of the distraction regenerate at the end of the active distraction period, the contralateral side was used as a control. The distraction regenerates were analyzed by plain radiography, micro-computed tomography and histological examination. Results. The rhBMP-2 appears to promote bone formation at both rapid and routine distraction rates, but no statistically significant difference was observed between the BMP injection sides of the rapid and routine distraction groups. Conclusion. This study indicates that rhBMP-2 can enhance bone formation at both routine and rapid distraction rates. Addition of rhBMP-2 seems able to compensate for the adverse effects of rapid distraction rates on mandibular distraction osteogenesis.

Key words: Bone morphogenetic proteins; Mandible; Osteogenesis, distraction; Rabbits

Introduction

Distraction osteogenesis is a method of producing new bone directly from an osteotomy site by gradual traction of the divided bone fragments. Since the first clinical report on the use of distraction osteogenesis to lengthen the human mandible in 1992, it has become a widely accepted approach to the treatment of severe craniofacial deformities. However, one of the major disadvantages of this technique is the lengthy course of treatment required for distraction and consolidation, which may result in pin tract soft-tissue infection, bone infection, and psychological problems. Consequently, both surgeons and patients would welcome any technical improvement that can speed up the treatment process.

The rate of distraction is the extent to which the bone fragments are stretched apart per day. The ideal rate for lengthening is widely accepted to be around 1.0 mm per day. Slow distraction tends to result in premature union, whereas rapid distraction may delay bone union or even give rise to fibrous union. In rabbit mandibular distraction models different distraction protocols have been used: 1.0 mm once daily, 0.5 mm twice daily, 0.18 mm twice daily, and 0.9 mm once daily, all of which are capable of leading to complete bone healing. At distraction rates as rapid as 1.5 mm twice daily, 1.0 mm twice daily and 2.0 mm once daily, unreliable bone ossification has been noted.

Bone morphogenetic proteins (BMPs) are among the most potent factors in bone development and healing, and have an important role in regulating bone and cartilage formation during distraction osteogenesis in long bones and in the mandible. The application of recombinant human bone morphogenetic protein-2
Bone morphogenetic protein on mandibular distraction osteogenesis has been reported in only a few publications. Both rhBMP-2 and rhBMP-4 were shown to enhance bone formation in the early stage of long bone and mandible distraction. On the other hand, application of rhBMP-7 during distraction osteogenesis has been associated with conflicting results. No study has compared the effect of rhBMPs on distraction regenerates under routine and rapid rates of distraction.

This study therefore aimed to evaluate the effect of rhBMP-2 on mandibular distraction at routine and rapid distraction rates, and thus determine the feasibility of compensating for the effects of increased distraction rates while maintaining the quality of the regenerate.

Materials and methods

Animal care

The animal experiment protocol was approved by the Committee on the Use of Live Animals for Teaching and Research, the University of Hong Kong. Eighteen adult New Zealand white rabbits (3.0-3.8 kg) were used. For this purpose, the rabbits were kept in a dedicated animal holding facility under veterinary supervision in the Laboratory Animal Unit of the Faculty of Medicine, the University of Hong Kong.

Surgical procedure and postoperative care

A standardized surgical procedure used in our previous study was performed on all the rabbits. Briefly, after the administration of preoperative antibiotic and analgesic (30 mg/kg long-acting oxytetracycline and 0.03 mg/kg buprenorphine), each rabbit was anesthetized by intramuscular injections of ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (1 mg/kg). With the rabbit's head hyper-extended, the skin was incised along the inferior border of both sides of the mandibular body. The platysma was dissected and the periosteum was reflected laterally. With a small bur, on both sides straight body osteotomy cuts were performed immediately anterior to the first premolar root. The custom-designed bone borne distractors were adapted on both sides of the mandible along a plane perpendicular to the osteotomy cut and fixed by 2-mm diameter titanium screws. The periosteum, muscle, and skin were repositioned and closed using 3-0 silk sutures.

After the operation, an antibiotic (long-acting oxytetracycline 30 mg/kg) was administered intramuscularly, twice per week for 2 weeks. For pain relief, buprenorphine (0.03 mg/kg) was administered subcutaneously twice daily for 10 days. Each animal remained under close observation by a veterinary technician until it regained consciousness. The clinical condition, weight and food consumption of the animals were monitored.

Distraction procedure

Eighteen rabbits were randomly assigned to two experimental groups, each with nine rabbits. After a 5-day latency period, bilateral distraction was activated at 0.9 mm once daily for 12 days in the routine rate group, and at 2.7 mm once daily for 4 days in the rapid rate group. An 1.08 mg rhBMP-2 in phosphate buffer (Institute of Basic Medical Sciences, Beijing, China) was injected into one side of the distraction regenerate at the end of the active distraction period, whereas the contralateral side was used as a control. Three rabbits in each group were sacrificed after 1, 2, and 4 weeks of the consolidation period, respectively.

Assessment methods

Plain radiography

Each mandibular specimen was placed on an occlusal film with the lingual side touching the film. Plain radiography was performed by an Orthoralix 9200 X-ray machine (Gendex, Des Plaines, USA) under standard conditions (50 KV, 16 mAs).

Micro-computed tomography

After plain radiographic examination, the distracted tissue regenerate and a 2-5 mm section of the neighboring normal bone in the distracted mandible were harvested. The specimens were subjected to morphological and quantitative examination by a µCT20 scan machine (Scano Medical AG, Bassersdorf, Switzerland). Each harvested specimen was placed into a 17-mm diameter sample holder with the sagittal plane vertical to the X-ray tube. For each specimen, 120-140 cross-sectional scans with a slice increment of 100 µm were made.

The standardized method of quantitative assessment described in our previous study was performed. Briefly, the serial scanned images for each specimen were inspected on the computer. On each scanning image, the total area of the distraction regenerate was outlined as the region of interest (ROI). The bone volume fraction (the
ratio between bone volume and total volume, BV/TV) within the ROI on each section was calculated individually and a mean value of BV/TV for the total regenerate was obtained by pooling from all the scanned sections within the distraction gap. To determine the threshold, all the ROIs on each specimen were subjected to an adaptive procedure in which the bone fraction was determined for a range of thresholds. The optimal threshold (120) was defined at the minimum change of bone fraction.

**Histology**

After the micro-computed examination, the samples were decalcified in a solution of 14.5% ethylenediaminetetraacetic acid buffered (pH 7.2) at room temperature. The decalcified specimens were processed and embedded in paraffin wax. Sections of 5 µm in thickness were cut longitudinally in the axial plane with a microtome and stained with hematoxylin and eosin for light microscopy.

**Statistical test**

The intra-individual controls were compared by paired t test, and groups at different distraction rates were compared by the two sample t test with the Statistical Package for the Social Sciences for Windows (SPSS Inc., Chicago[IL], USA). A probability of 0.05 or less was considered significant.

**Results**

**Clinical examination**

All 18 rabbits completed the experimental process uneventfully. None of the animals experienced postoperative complications, and the distractors remained stable till the day of sacrifice. Both sides of the mandible were lengthened symmetrically, and obvious crossbite and overgrowth of the lower incisor developed in all the rabbits.

**Plain radiographic examination**

In the routine distraction group, after 1 week of consolidation two of the three rabbits sacrificed still demonstrated incomplete union at the central area of the distraction regenerate on the control side. Whereas, confluent radio-opacity across the distraction gaps of the BMP injection side was noted in all three cases. In the corresponding rapid distraction group, radio-opaque streaks were observed in both the BMP injection and control sides, but partial union only was noted in two of the three cases of the BMP injection side.

At week 2 of consolidation, the osteotomy site demonstrated bony continuity in both the BMP injection and control sides of the routine distraction group, and the radio-density of BMP injection side seemed to be greater. In the rapid distraction group, complete bone union was observed in two of the three cases on the BMP injection side, but the control side still demonstrated obvious non-union in the central area.

At week 4 the radiographic images of the BMP injection sides of both the routine and rapid distraction groups appeared similar. When compared with the control sides, the radio-density in the distraction gap was greater and clear corticalization was only observed on the BMP injection side. On the control side, bone union was complete in all the cases, but corticalization was not clearly seen.

**Micro-computed tomography**

Distraction regenerate in the serial computed tomographic (CT) images demonstrated continuous ossification with gradually increasing mineralization from the center to the edges. Bone formation and remodeling of the distraction regenerate on the BMP injection sides (in both the routine and rapid distraction groups) were more obvious than on the control sides at all observation time-points.

At week 1 of consolidation, obvious new bone formation was seen in all mandibular samples of the experimental animals. More bone formation was noted on the BMP injection side of both the normal and rapid distraction groups than on the control side (Figures 1a-d).

At week 2 of consolidation, new bone formation was more obvious in all the mandibular samples (Figures 1e-h). The BMP injection side of both the normal and rapid distraction groups displayed more new bone than on the control side.

Early corticalization was only observed on the BMP injection side of the normal distraction group. The peripheral bone became denser and the marrow cavity became evident, although it was not yet very clearly defined (Figure 1f).

At week 4 of consolidation, the CT images of the BMP injection side of the normal and rapid distraction groups were quite similar; advanced remodeling was noted, and
obvious corticalization and marrow cavity formation were clearly seen (Figures 1j and l). On the control side, multifocal bony defects were still obvious (Figures 1i and k).

Quantitative analysis of the distraction regenerate showed that bone volume of the BMP injection sides was higher than that on the control sides, in both the routine and rapid distraction groups (Figures 2a and b).

Difference in bone volume between the groups became statistically significant at 2 weeks and 4 weeks of consolidation. When the bone volume of the BMP injection sides was assessed, the routine distraction group tended to have higher values than the rapid distraction group, but the differences were not statistically significant (Figure 2c).

### Histology

Corresponding to the findings demonstrated by the micro-CT study, histological examination showed that bone formation and remodeling on the BMP injection sides was more advanced than on the control sides.

At week 1 of consolidation, in the routine distraction group the distraction regenerate of both the experimental and control sides was composed of primary trabeculae and loose fibrovascular stroma; fibrous tissue still being obvious in the central area. On the control side, there were still a few small cartilage islands at the central area close to the periosteum. In the rapid distraction group, most of the distraction gap was bridged by fibrous tissue; new bone had generated at the edges of the distracted gap, and also at the medial surface of the distraction regenerate.
(by peristonic reaction). At week 2, bone formation and remodeling was more advanced in BMP injection sides of both the routine and rapid distraction groups, and complete bony union was observed in the central area of the distraction regenerate. On the control sides, focal fibrous tissue was obvious in the routine distraction group, and even more so in the rapid distraction group.

At week 4, on the BMP injection sides of both groups, bone remodeling was evident; increased thickness of the new cortex and bone marrow cavity formation were clearly seen. On the control sides, though the distraction gap was completely united, corticalization and marrow cavity formation were not yet evident in the routine distraction group. On the control side in the rapid distraction group, the presence of fibrous tissue was obvious.

**Discussion**

Varying the rate of distraction significantly influences the outcome of distraction osteogenesis. A higher rate of distraction may lead to poor bone quality and a high incidence of soft tissue complications. Li et al. investigated the angiogenic response to different distraction rates and demonstrated that vascularization in the central fibrous zone was maximally stimulated at distraction rates of 0.7 to 1.3 mm/day. A slow rate of distraction at 0.3 mm/day does not maximally stimulate angiogenesis in the distraction regenerate, and a high rate (2.7 mm/day) appeared to impair this response. A study in our department demonstrated that change in the mechanical environment resulting from different rates of distraction leads to different expression of the BMP-2 and -4. The biological environment created by distraction at a routine rate (0.9 mm/day) is superior to that ensuing with a rapid rate (2.7 mm/day) during the early stage of consolidation.

From the clinical perspective, most surgeons in maxillofacial and orthopedic surgery would wish to reduce the treatment course of distraction osteogenesis. Reducing the consolidation period or increasing the distraction rate are possible options. The present study attempted to facilitate an accelerated distraction rate with the injection of rhBMP-2, and compare the ensuing bone ossification process at routine and rapid distraction rates.

Use of rhBMP-2 (among the most potent of the BMP family) has been shown to promote bone regeneration and remodeling during the bone repair process in a variety of animal models. The development of the recombinant DNA techniques enables recombinant human BMPs to be produced reliably and in substantial quantities, which offers promise for hard tissue engineering.

A baseline study for this work conducted in our department demonstrated that the osteogenic activity of rhBMP-2 in mandibular distraction osteogenesis is dosage-dependent, and that a single injection of rhBMP-2 of 1.08 mg is just as effective as multiple injections. In the current study, a single injection of 1.08 mg rhBMP-2 was used at the end of the active distraction period. In reconstructive surgery, exogenous BMPs are normally delivered to the site of regeneration by the carrier matrix.
The matrix is supposed to be capable of retaining the BMP at the site of implantation and controlling its release to the tissues. Although a matrix is often used together with BMP, there is no biological indication for this practice. If enough BMP is applied, bone is formed in the absence of a matrix. Bony reconstruction of a surgical defect is different from the distraction regenerate, because the latter provides a richer source of osteoprogenitor cells that are essential for bony regeneration. We delivered rhBMP-2 directly to the distracted area by percutaneous injection without a matrix in the present study.

One week after the completion of active distraction, obvious signs of new bone formation were seen in all mandibular samples. In both the routine and rapid distraction groups, the images obtained by all assessment methods suggested that the bone ossification on the BMP injection sides was superior to that on the control sides, but the differences were not statistically significant. Histological study showed a few small islands of cartilage on the control sides of the normal distraction group, but bone formation during distraction osteogenesis was primarily through intramembranous ossification. At week 2, bone formation in the BMP injection sides was significantly enhanced in both the routine and rapid distraction groups. Bone volume of the experimental sides was significantly greater than that of the control sides. At week 4, the BMP-treated samples at both routine and rapid distraction rates were similar; bone union was complete in the central area, and the cortex and marrow cavity architecture could be distinguished. On the injected side, although the ossification process seemed superior to that encountered with rapid distraction, no statistically significant difference was observed.

In conclusion, this study indicates that rhBMP-2 can enhance bone formation at both routine and rapid distraction rates. The addition of rhBMP-2 seems able to compensate for the rapid distraction rates in mandibular distraction osteogenesis.

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References

Zheng and Cheung