Available online at www.sciencedirect.com
ScienceDirect
British Journal of Oral and Maxillofacial Surgery 44 (2006) 487–494
BRITISH Journal of Oral and Maxillofacial Surgery
www.bjoms.com

Evaluation of recombinant human bone morphogenetic protein-2 in mandibular distraction osteogenesis in rabbits: Effect of dosage and number of doses on formation of bone

Li Wu Zheng [a], Martin C.M. Wong [b], A. Bakr M. Rabie [c], Lim K. Cheung [a,*]

[a] *Discipline of Oral & Maxillofacial Surgery, Faculty of Dentistry, Prince Philip Dental Hospital, The University of Hong Kong, 34 Hospital Road, Hong Kong SAR, China*
[b] *Industrial Centre, The Hong Kong Polytechnic University, Hong Kong SAR, China*
[c] *Discipline of Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China*

Accepted 7 September 2005
Available online 17 October 2005

Abstract

We evaluated the dose- and time-dependent response of recombinant human bone morphogenetic protein-2 (rhBMP-2) to the formation of bone in mandibular distraction osteogenesis. Twenty-one adult white New Zealand rabbits (3.0–3.8 kg) were used to establish the mandibular distraction model, 18 of which completed the experiment. Eight rabbits were given rhBMP-2 360 μg and eight 1080 μg; two were given no rhBMP-2. The fluids were injected into the regenerating bone at three different time sequences (days 5, 8, and 11 of active distraction; days 5 and 11 of active distraction; and day 11 of active distraction alone). After four weeks of consolidation, the specimens were harvested and examined radiographically by micro-computed tomography (micro-CT), and histologically. The formation and remodelling of bone in distraction osteogenesis was significantly increased by the addition of rhBMP-2, and the increase was dose-dependent. There was no significant difference between different dosage regimens. A single injection of rhBMP-2 at the end of the distraction phase was as effective as multiple injections.

Keywords: BMP; Mandible; Osteogenesis; Distraction

Introduction

Distraction osteogenesis is a way of producing new bone directly from the osteotomy site by gradual distraction of the divided bony fragments. Since the first clinical report on the use of distraction osteogenesis to lengthen the human mandible in 1992,[1] it has become widely accepted in the treatment of severe craniofacial deformities. However, one of its major disadvantage is the long course of treatment required for distraction and ossification, which may result in infection of soft tissue and bone and psychological problems. Both surgeons and patients would welcome any technical improvement that speeds up treatment.

Urist discovered that demineralised bone matrix was capable of inducing formation of bone in intramuscular sites.[2] The development of recombinant DNA enables recombinant bone morphogenetic proteins (rBMPs) to be produced reliably in large quantities, and this offers promise for hard tissue engineering. Among the most potent of the BMPs is rhBMP-2, and it is capable of promoting regeneration and remodelling of bone during the repair in various animal models. This formation of new bone induced by BMPs is dose-dependent.[3–6] As well as the dose, optimal delivery and controlled release systems are important. Although a matrix has been often used, formation of bone can be achieved without a matrix if enough BMP is applied.[7,8]

* Corresponding author. Tel.: +852 2859 0267; fax: +852 2559 9014.
E-mail address: lkcheung@hkucc.hku.hk (L.K. Cheung).

0266-4356/$ – see front matter
doi:10.1016/j.bjoms.2005.09.005

Endogenous BMPs are also important local agents that regulate formation of bone and cartilage in distraction osteogenesis in long bones[9–11] and mandible.[12,13] The use of rhBMPs to promote bony induction and ossification in distraction osteogenesis has been reported in only a few publications. At a rapid distraction rate of 2.0 mm/day, rhBMP-2 and rhBMP-4 may improve formation of bone at the early stage of distraction of long bones and mandible.[7,14] However, rhBMP-7 has given conflicting results in distraction osteogenesis at a normal distraction rate. Hamdy et al. reported that rhBMP-7 showed a dose-dependent response in the lengthening of long bones.[15] Rabbits that were given a high dose (2000 μg) of rhBMP-7 recorded high values in densitometric and histomorphometric measurements of bony mass as well as in biomechanical testing. However, the results were not significant. Mizumoto et al. reported promising results from the application of rhBMP-7 for lengthening of long bones in rats.[16]

The correlation between dose of rhBMP and time of giving it, and regeneration of bone by distraction osteogenesis is not clear. In this study, we aimed to clarify the relation between the dose and time of injection of rhBMP-2 and the formation of bone in distraction osteogenesis in mandibles of rabbit.

Materials and methods

Animal care

The experimental protocol was approved by the Committee of the Use of Live Animals for Teaching and Research, The University of Hong Kong. Twenty-one adult New Zealand white rabbits (3.0–3.8 kg) were used. The rabbits were kept in a dedicated animal house under veterinary supervision in the Laboratory Animal Unit of the Faculty of Medicine, The University of Hong Kong.

Distractor

Bone-borne external distractors, weighing 3.7 g each, were custom-designed and made in medical-grade stainless steel (Fig. 1). A full turn of the activation rod produced a distraction of 0.45 mm between the fixation arms. The full distraction capacity of the distractor was 15 mm. The anterior and posterior fixation arms on the mandible were fixed by two or three titanium screws 2 mm in diameter.

Operation

A standard operation was done. The animals were given preoperative doses of an antibiotic and an analgesic (long acting oxytetracycline 30 mg/kg and buprenorphine (Temgesic) 0.03 mg/kg), and were anaesthetised by intramuscular injection of ketamine 35 mg/kg, xylazine 5 mg/kg, and acepromazine 1 mg/kg. The skin was incised along the inferior border of the mandibular body with the rabbit's head hyperextended. The platysma was dissected and the periosteum raised from the mandible and reflected laterally to identify the mental nerve, which was located immediately anterior to the first premolar tooth. A straight body osteotomy cut was made with a small Lindermann bur immediately anterior to the first premolar root on one side of the mandible. The custom-made, bone-borne distractor was placed 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 with 3/0 silk sutures.

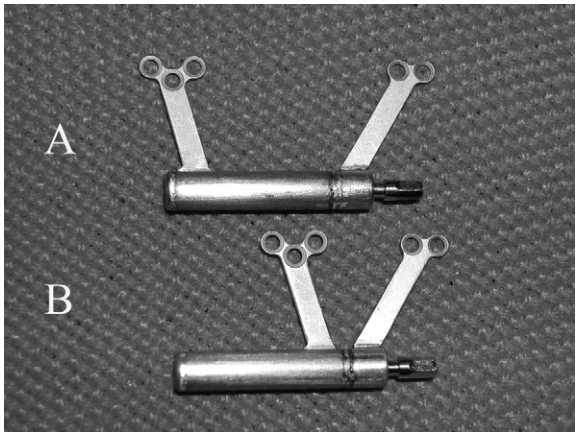

Fig. 1. The custom-made distractor is fully open (15 mm horizontally) (A) compared with when closed (B).

Postoperative care

After the operation, an antibiotic (long-acting oxytetracycline 30 mg/kg) was given intramuscularly twice a week for two weeks. For pain relief, buprenorphine 0.03 mg/kg was given subcutaneously twice a day for 10 days. Each animal was observed closely by a veterinary technician until it regained consciousness. The clinical condition, weight, and food consumption of the animals were monitored. Distraction produced a gradually increasing malocclusion. To alleviate any gingival ulceration, the incisors were ground down at the end of active distraction and every two weeks thereafter.

Distraction

After a 5-day latency period, active distraction was begun at a rate of 0.9 mm once a day for 11 days. The distractors were kept in place until the rabbits were killed. Different doses of rhBMP-2 (Institute of Basic Medical Science, Beijing, China) or phosphate-buffered saline (PBS) without rhBMP-2 were injected into the distracted material at different times (Table 1). The rabbits were killed 28 days after the completion of active distraction by an intravenous injection of sodium pentobarbitone (60 mg/kg). The distracted side of the mandible was harvested and fixed in 10% neutral phosphate-buffered formalin.