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The influence of Bone Morphogenic Protein-2 on the consolidation phase in a distraction osteogenesis model

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ABSTRACT

We asked whether locally applied recombinant-Bone Morphogenic Protein-2 (rh-BMP-2) with an absorbable Type I collagen sponge (ACS) carrier could enhance the consolidation phase in a callotasis model. We performed unilateral transverse osteotomy of the tibia in 21 immature male rabbits. After a latency period of 7 days, a 3-weeks distraction was begun at a rate of 0.5 mm/12 h. At the end of the distraction period (Day 28) animals were randomly divided into three groups and underwent a second surgical procedure: 6 rabbits in Group I (Control group; the callus was exposed and nothing was added), 6 rabbits in Group II (ACS group; receiving the absorbable collagen sponge soaked with saline) and 9 rabbits in Group III (rh-BMP-2/ACS group; receiving the ACS soaked with 100 μg/kg of rh-BMP-2, Inductos®, Medtronic). Starting at Day 28 we assessed quantitative and qualitative radiographic parameters as well as densitometric parameters every two weeks (Days 28, 42, 56, 70 and 84). Animals were sacrificed after 8 weeks of consolidation (Day 84). Qualitative radiographic evaluation revealed hypertrophic calluses in the Group III animals. The rh-BMP-2/ACS also influenced the development of the cortex of the calluses as shown by the modified radiographic patterns in Group III when compared to Groups I and II. Densitometric analysis revealed the bone mineral content (BMC) was significantly higher in the rh-BMP-2/ACS treated animals (Group III).

Introduction

Induction of osteogenesis by means of callotasis[2] (distraction osteogenesis) is useful in the treatment of congenital limb deformities, leg length discrepancies, fracture nonunion, and bone loss after trauma or tumour resection.[11,17–19,23,28,29,40] First described in 1905 by Codivilla for the treatment of limb length discrepancy[8] the technique of distraction osteogenesis (DO) and its clinical applications were extensively studied and described by Ilizarov[17,18] in the 1980s. DO is a bone-regenerative process in which gradual distraction yields two vascularized bone surfaces, from which new bone is formed. The basic principle of the callotasis technique includes performing an osteotomy before gradually distracting the two bone segments using an external device. New bone tissue is generated in the gap between the two bone segments that are progressively distracted.

Distraction osteogenesis can be divided into three temporal phases: latency, distraction and consolidation. After distraction, the long duration of the consolidation phase (requiring prolonged external fixation) can be a cause of considerable morbidity for the patient.[11,30,34] In clinical practice there is a need for enhancing consolidation during distraction osteogenesis as it may contribute to reduced morbidity.[3]

The molecular signals that drive the regenerative process of DO are quite similar to those characterising fracture repair and include the pro-inflammatory cytokines, the transforming growth factor beta superfamily, and angiogenic factors.[1] Various studies have reported that amongst growth factors, bone morphogenetic proteins (BMPs) may play a central role in the molecular signalling cascade leading to bone regeneration and remodelling in a DO procedure.[5,13,32,42] Bone morphogenic proteins (BMP) are also recognised as the most potent of the osteoinductive factors.[4,6,14,15,38,45,47,48] They play a crucial role in the process of bone regeneration and consolidation during DO.[5,32,41,51] Few studies have shown recombinant-BMP-7 and -2 (rh-BMP-7 and rh-BMP-2) could enhance the consolidation phase in long bone DO models.[25,27,36] Others showed rh-BMP-7 failed to enhance bone

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consolidation in similar animal models.[13,35] The specific reasons of success or failure of these similar drug-testing animal models are not clearly understood. It seems that the phase (liquid or solid), the quantity and the timing of application of the rh-BMP may influence the bone consolidation process.[13,35,36]

Rh-BMP-2 has been shown to accelerate bone regeneration in both mandibular and tibial distraction models.[7,25,36,46] In a previous study (same experimental model) we were able to show that a single application of rhBMP-2/absorbable collagen sponge (ACS) on the day of osteotomy could stimulate the bone regeneration process in a dose-dependent manner.[36] The same study also suggested that using a dose of 200 μg/kg of rh-BMP-2/ACS will result in premature bone fusion in most cases. With a lower dose (100 μg/kg of rh-BMP-2/ACS) the entire desired distraction could be performed in most of the cases before the consolidation phase began.

In the present study we asked whether a single dose of 100 μg/kg of rh-BMP-2/ACS applied at the end of the distraction period (thus eliminating the risk of premature bone fusion) could enhance the consolidation of the bone regenerate in a rabbit model of tibia lengthening. Consolidation was assessed by radiographic and densitometric parameters.

Materials and methods

We randomly selected 21 male New Zealand white rabbits that weighed 3.2–3.5 kg. The animals were then randomly separated into three groups as follows: 6 rabbits in Group I (control group, without rh-BMP-2 or ACS), 6 rabbits in Group II (ACS group receiving the absorbable collagen sponge soaked with saline) and 9 rabbits in Group III (rh-BMP-2/ACS group, receiving the ACS soaked with 100 μg/kg of rh-BMP-2). Animals were assigned to each group before the surgical procedure. The experimental protocol was approved by the Comité d'Ethique de l'Ecole Vétérinaire de Maison Alfort and by the Comité d'Expérimentation Animale de l'Université Paris 7 (Laboratoire B2OA).

For each surgical procedure (two per animal) the 21 rabbits were premedicated with an intramuscular injection of 0.1 mL/kg of xylazine (ROMPUN® 2%, Ponteaux, France) and 0.1 mL/kg of ketamine (IMALGENE 1000®, Lyon, France). We induced anaesthesia by an intramuscular injection of ketamine (20–40 mg/kg) and of xylazine (3–5 mg/kg) 15 min later. The anaesthesia was maintained during surgery using a facemask with isoflurane (FLUOTHANE®, 5%, Avonmonth, UK). Oxygen was supplied during the surgical procedure through the facemask. For radiographic and dual energy X-ray absorptiometry (DEXA) examinations animals were anaesthetized with an intramuscular injection using 0.1 mL/kg of xylazine (2%) and 0.1 mL/kg of ketamine.

First we washed and prepared the left leg of the animal for sterile isolation. We made a skin incision on the lateral aspect of the left tibia. We retracted the lateral tendons and inserted the most distal self-tapping screw (1.6 mm diameter) perpendicular to the longitudinal axis of the tibial shaft. We then positioned an M-103 external fixator (Orthofix, Inc., Verona, Italy) onto the screw and applied the most proximal screw through the fixator clamp. The remaining two screws were inserted using the fixator clamps as guides. The fixator clamps were tightened over the screws. We then incised and retracted the periosteum at the level of the two inner screws (between the inner screws) for the tibial shaft to be exposed using specific retractors. We then performed a mid-shaft osteotomy. The transverse osteotomy was made between the two inner screws at the level of the tibiofibular junction using a low speed 1 mm drill under constant saline irrigation. The fixator was maintained in place during the osteotomy. We repositioned the periosteum and deep tissues and closed the wound using an absorbable suture.

After a latency period of 7 days postoperatively, the distraction was started at a rate of 0.5 mm/12 h for 21 days.

After the distraction period all 21 rabbits experienced a second surgical procedure, at Day 28. We made a skin incision (2 cm) on the medial aspect of the left tibia at the level of the two inner screws. The tendons and muscles were retracted and the bone regenerate filling the distraction gap was exposed with great care not to damage the tissues. Animals in Group I (control group) did not receive any supplementary material, the periosteum was repositioned and the wound closed using an absorbable suture (blank procedure). Animals in Group II received ACS soaked with saline. Animals in Group III received the ACS soaked with 100 μg/kg of rh-BMP-2 (Inductos®, Medtronic). A minimum of 15 min was held with the ACS soaked with rh-BMP-2 before application as recommended by Medtronic. In Group II and Group III the ACS was wrapped around the tissue regenerate filling the distraction gap. The periosteum and deep tissues were repositioned over the ACS and the skin incision was closed using an absorbable suture.

The operated left leg was dressed after each surgical procedure. The analgesia was maintained postoperatively using a Fentanyl (DUROGESIC®, Issy les Moulineux, France) patch. The dressing was changed every day during the first week. We subcutaneously injected antibiotics (0.3 mL of trimetoprim, SEPTOTRIL®, Paris, France) every day during the first postoperative week. Animals were housed in separate cages with free access to food and water. Activity and weightbearing were unrestricted immediately after the operation.

All 21 rabbits were sacrificed at day 84, after 2 months of consolidation.

Radiographic screening was used to compare between groups the development and maturation of the distracted callus. Anteroposterior (AP) and lateromedial (LM) plain radiographs were taken 28, 42, 56, 70 and 84 days after the first operation under general anaesthesia. The radiographic technical parameters were 43 kV, 400 mA, and 32 ms. The AP and LM radiographs included the ankle and the knee joints. Qualitative and quantitative evaluations of the callus were performed for each radiograph. The percentage area of the distraction gap occupied by new bone tissue was scored by three independent and blinded observers according to the methods reported by Kirker-Head et al.[21,22] and Yasko et al.[50] This percentage area of distraction gap occupied by new bone was graded from 0 to 5 (Table 1). In the Yasko and Kirker-Head grading scale system we decided to add a grade 6 as in Group III we identified several animal showing hypertrophic calluses with the bone regenerate occupying more than 100% of the distracted gap (Table 1). An average score from the three observers was recorded for each radiograph. For final analysis, the means of the scores of all radiographs from the same time point were calculated and recorded. Qualitative analysis consisted of analysing the presence of cortical continuity on the medial and lateral aspects of the regenerate within the distraction gap at each timepoint.

We assessed the bone mineral content (BMC) of the regenerate region using DEXA (pDEXA Norland XR-36, Medical System, Fort Atkinson, WI). Measures were made under general anaesthesia every two weeks starting from the end of distraction (Days 28, 42,

Table 1
Bone formation grading score for radiographic quantitative analysis.

Grade	Total distraction gap filled by new bone
0	No formation
1	<25%
2	≥25% to <50%
3	≥50% to <75%
4	≥75% to <100%
5	100% formation
6	>100%/hypertrophic