Bone 58 (2014) 26–32

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Manipulation of anabolic and catabolic responses with bone morphogenetic protein and zoledronic acid in a rat spinal fusion model

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ARTICLE INFO

Article history:
Received 17 February 2013
Revised 25 September 2013
Accepted 27 September 2013
Available online 5 October 2013

Edited by: Thomas Einhorn

Keywords:
Bone morphogenetic protein
Zoledronic acid
Spine fusion
Rat model

ABSTRACT

Bone fusion involves a complex set of regulated signaling pathways that control the formation of new bone matrix and the resorption of damaged bone matrix at the surgical site. It has been reported that systemically administering a single dose of zoledronic acid (ZA) at the optimal time increases the strength of the bone morphogenetic protein (BMP)–mediated callus. In the present study, we aimed to investigate the effect of BMP-2 and ZA in a rat spinal model. Sixty-seven rats were divided into 6 groups: group I (n = 11) animals were implanted with a carrier alone, group II (n = 12) animals were implanted with a carrier and a subcutaneous injection of ZA was administered 2 weeks after surgery, group III (n = 12) animals were implanted with a carrier containing 1 μg of rhBMP-2, group IV (n = 12) animals were implanted with a carrier containing 1 μg of rhBMP-2 and a subcutaneous injection of ZA was administered 2 weeks after surgery, group V (n = 10) animals were implanted with a carrier containing 3 μg of rhBMP-2, and group VI (n = 10) animals were implanted with a carrier containing 3 μg of rhBMP-2 and a subcutaneous injection of ZA was administered 2 weeks after surgery. The rats were euthanized after 6 weeks, and their spines were explanted and assessed by manual palpation, radiography, high-resolution micro-computerized tomography (micro-CT), and histologic analysis. The fusion rates in group VI (60%) were considerably higher than those in the groups I (0%), II (0%), III (12.5%), IV (20.8%), and V (35%), ($P < 0.05$). Additionally, the radiographic scores of group VI were higher than those in the other groups, ($P < 0.05$). In micro-CT analysis, the tissue and bone volumes of the callus were significantly higher in group VI than those in the other groups, ($P < 0.05$). The trabecular number was significantly higher and the trabecular spacing was significantly lower in group VI than those in the other groups, ($P < 0.05$). The combination of rhBMP-2 and ZA administered systemically as a single dose at the optimal time was efficacious in our rat spinal fusion model. Our results suggest that this combination facilitates spinal fusion and has potential clinical application.

Introduction

Spinal arthrodesis is a fundamental treatment option for spinal pathologies and one of the most common spinal procedures, with more than 200,000 surgeries performed in the United States each year [1]. This procedure is the gold standard for treatment of degenerative and traumatic spine diseases associated with severe neck or back pain, and sometimes, neurologic problems. In this procedure, bone grafts are used to restore mechanical stability to the affected spinal segment by providing bridging bone between vertebrae. Because successful bone fusion between unstable spinal segments leads to pain relief and neurologic recovery, the efficacy of this procedure has gained wide acceptance, and the number of these types of surgery has increased annually with the increase in the aged population [2–5].

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β superfamily [6], and are powerful osteoinductive molecules. An in vitro study has shown that BMPs work by stimulating pluripotent mesenchymal cells to differentiate into osteoblasts, thereby producing a bone matrix. BMPs also are considered to promote new osteoclast formation since they stimulate the production of receptor activator of nuclear factor kappa-B ligand (RANKL) osteoblasts and help ensure mature osteoclast survival; therefore, BMPs participate in bone matrix resorption [7,8].

The osteoinductive effects of recombinant human BMPs (rhBMPs) for spinal fusion have been shown in animal models and clinical trials [9–13]. Although BMPs are approved for clinical use, clinical trial results have shown that high doses are required to induce adequate bone fusion because of the following reasons: (1) solubility of the molecules, (2) easy diffusion of the molecules away from the fusion site, and (3) in vivo inactivation [14]. In addition, BMPs are expensive; therefore, their usefulness may be limited by their expense. As a result, a number of strategies are being developed to provide a safer, less expensive, and more efficacious spinal fusion using rhBMP.

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http://dx.doi.org/10.1016/j.bone.2013.09.021

Bisphosphonates mediate complex effects on bone: they primarily show anticatabolic effects. Circulating bisphosphonates bind to bone mineral. When the bone is resorbed by osteoclasts, the osteoclasts undergo apoptosis [15,16]. Bisphosphonates have improved the clinical outcomes of osteoporosis, Paget's disease, and metastatic bone disease [17–19]. Zoledronic acid (ZA), the most potent bisphosphonate, can be administered as a single systemic dose. In a rabbit spinal fusion model, systemic ZA administration increased fusion mass size, bone mineral content, and fusion rate [20]. On the other hand, long-term bisphosphonate administration leads to decreased bone formation, which is thought to result from uncoupling of the balance between osteoclastic and osteoblastic activity [21]. Following the use of bisphosphonates in millions of patients in clinical practice, some unexpected possible adverse effects have been reported, including osteonecrosis of the jaw, and atypical femur fractures [22].

Although many factors influence bone fusion modification, the ultimate result is determined principally by the balance between anabolic osteoblast and catabolic osteoclast responses. We previously reported that the synergic effect of rhBMP-2 and ZA administered systemically as a single dose at the optimal time was efficacious for fracture repair, and significantly enhanced bone fusion in a rat femoral fracture model [23]. However, the mechanism of these effects in spinal bone fusion remains unclear. We also previously tested the osteogenic activity of rhBMP-2 in a rodent spinal fusion model, and tried to provide a safer, less expensive, and more efficacious bone fusion using rhBMP-2 [24]. The purpose of the present study was to elucidate the synergic effect of rhBMP-2 and ZA administered as a single systemic dose for spinal fusion and to examine its feasibility for clinical application by using a rat spinal fusion model.

Materials and methods

Preparation of matrices

MedGEL (MedGEL, Kyoto, Japan) is a biodegradable gelatin hydrogel scaffolding for cellular attachment [25]. The MedGEL was cut using a scalpel into 5 mm × 10-mm strips. To prepare MedGEL incorporating rhBMP-2 (Peprotech, Rocky Hill, NJ), 100 μL of phosphate-buffered saline solution (PBS, pH 7.5) containing 1 μg or 3 μg of rhBMP-2 was dropped onto MedGEL and left overnight at 4 °C on an Eppendorf tube prior to implantation. Similarly, 100 μL of rhBMP-2-free PBS was dropped onto MedGEL to obtain the rhBMP-2-free empty MedGEL.

Study groups

Sixty-seven male Sprague–Dawley rats (16–18 weeks old; CLEA Japan, Inc., Tokyo, Japan) were divided into 6 groups: group I (n = 11) animals were implanted with MedGEL alone, group II (n = 12) animals were implanted with MedGEL and a subcutaneous injection of ZA was administered 2 weeks after surgery, group III (n = 12) animals were implanted with MedGEL containing 1 μg rhBMP-2, group IV (n = 12) animals were implanted with MedGEL containing 1 μg of rhBMP-2 and a subcutaneous injection of ZA was administered 2 weeks after surgery, group V (n = 10) animals were implanted with MedGEL containing 3 μg of rhBMP-2, and group VI (n = 10) animals were implanted with MedGEL containing 3 μg of rhBMP-2 and a subcutaneous injection of ZA was administered 2 weeks after surgery. ZA was prepared in sterile saline from commercial 4-mg vials (Novartis Pharma KK, Tokyo, Japan) and administered as a single subcutaneous injection of 0.1 mg/kg. The optimal time for administering systemic ZA is 2 weeks after surgery, as shown in a previous critical defect rat model [26]. Animals that were not scheduled to receive ZA were administered with control injections of saline.

Surgical technique for constructing L4–L5 posterolateral spinal fusion model

Approval was obtained from Oita University's Animal Research Committee prior to animal experimentation. A posterior midline incision was made on the skin. Next, 2 separate paramedian incisions were made at 3 mm from midline in the lumbar fascia, and the transverse processes were exposed. The transverse processes of the L4 and L5 were decorticated using a low-speed burr. Subsequently, MedGEL with or without rhBMP-2 was implanted on each side. The fascial and skin incisions were closed with a 3–0 absorbable suture. Immediately following surgery and on subsequent days, the rodents received analgesics (buprenorphine subcutaneously and paracetamol). The rodents were housed in separate cages and fed food and water ad libitum, and their conditions were monitored on a daily basis. The rats were humanly euthanized 6 weeks post operatively.

Manual assessment of fusion

Six weeks after implantation, explanted spines were manually tested for intersegmental motion by 3 blinded independent observers. The explanted lumbar spine was palpated gently, and lateral side-bending motion at the L4–L5 level was compared with the motion at the adjacent levels above (L3–L4) and below (L5–L6). The absence of motion was considered as successful fusion. Any motion detected between the transverse processes was considered a failure of fusion. The spine was designated as "not fused" if any of the 3 observers graded it as not fused. The spines were scored as either fused or not fused on both the right and left sides. The fusion rate then was calculated.

Radiographic analysis

The explanted spines obtained at the 6-week time point were radiographed using a Softex radiograph apparatus (Softex CSM-2; Softex, Tokyo, Japan) employing an HS Fuji Softex film (Fuji Film, Tokyo, Japan) at 45 cm with 30 kV and 15 mA for 20 s. Fusion between the L4 and L5 transverse processes in each rat was recorded as a percentage of the total area between the L4 and L5 that was filled with new bone. Three blinded independent observers scored the bone formation in each rat on a 5-point scale: 0 = no bone formation; 1 = bone filling less than 25% of the area; 2 = bone filling 25–50% of the area; 3 = bone filling 50–75% of the area; and 4 = bone filling 75–100% of the area. The spines were scored on both the right and left sides.

Micro-CT analysis

Next, the spines were scanned by micro-CT using SkyScan1172 (Bruker microCT, Kontich, Belgium) with voxel size of 20 μm. The data were collected at 100 kV and 100 μA, and reconstructed using the cone-beam algorithm. Each spine was set on the object stage and sample scanning was performed over 180° rotation with an exposure time of 105 ms. A cylindrical volume of interest with a diameter of 20 mm and a height of 27 mm was selected, which displayed the microstructure of the rat vertebra as comprising cortical and cancellous bone. Data analysis was performed using a CT Analyzer software (Bruker microCT). By using this software, the area from the top of the L4 transverse processes to the bottom of the L5 transverse processes, including the vertebrae, was analyzed. The spines were analyzed on both the right and left sides. In the 3-dimensional (3D) analysis, tissue volume (TV), bone volume (BV), trabecular thickness (Tb. Th), trabecular number (Tb. N), trabecular spacing (Tb. Sp), and bone volume fraction (BV/TV, %) were measured.

Histologic analysis

Six weeks after implantation, the spines were dissected, and the specimens were fixed in 40% ethanol, decalcified using standard 10% decalcifying solution HCl (Cal-Ex; Fischer Scientific, Fairlawn, NJ), washed with running tap water, and then transferred to 75% ethanol. Serial sagittal sections near the transverse processes were cut carefully