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COMP–angiopoietin-1 accelerates bone formation during distraction osteogenesis

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ABSTRACT

Introduction: During distraction osteogenesis, new and highly vascularized bone is formed, with angiogenesis preceding osteogenesis. We investigated the possibility that COMP–Ang1, an angiogenic factor, may facilitate bone formation.
Methods: Rats were divided into three groups. Control rats underwent tibial distraction without treatment. In the two remaining groups, BSA (100 μg) or COMP–Ang1 (100 μg) were injected transcutaneously into the center of the distraction zone. Using radiographic and histologic analyses, we assessed total bone volume, vascular density, and bone mineral density. Total RNA was prepared from regenerated bone and analyzed for osteogenic marker protein expression using real-time RT–PCR analysis.
Results: Bone formation in the distraction gap progressed more quickly in the COMP–Ang1-treated group than in the BSA-treated group. Histological findings and immunostaining of endothelial cells for factor VIII revealed that Comp–Ang1 group animals exhibited higher levels of vascularity. NanoCT and dual-energy X-ray absorptiometry analyses revealed increased new bone formation along capillaries in the COMP–Ang1 group compared with the BSA group. Runt-related transcription factor 2 and its target genes, including bone sialoprotein, type 1 collagen, osteopontin, and osterix, were significantly upregulated in the COMP–Ang1 group.
Conclusions: Our results are consistent with previous descriptions of the positive relationship between angiogenesis and osteogenesis. In addition, our results suggest the potential use of COMP–Ang1 as a therapeutic agent for treatment of distracted limbs by enhancing angiogenesis.

Introduction

Distraction osteogenesis is a surgical bone-lengthening procedure that is widely used to correct a variety of orthopedic disorders such as limb length discrepancies, bone defects, limb deformities, and fracture nonunions [1]. The principle allows for the formation of new bone after slow distraction of an osteotomy. The model of distraction osteogenesis consists of three stages: the latency stage after osteotomy and application of the external fixator, the distraction stage in which the separated bone ends are gradually and continuously distracted, and the consolidation stage. Although this procedure has revolutionized the treatment options for patients with orthopedic deformities and malformations, the main disadvantages are the considerable time needed to lengthen bone and the use of distraction devices for extended periods.

Like fracture healing, neovascularization is essential for distraction osteogenesis. New bone formation and remodeling during the distraction stage and subsequent consolidation period require an intense angiogenic response. Indeed, angiogenic response is associated closely with distraction osteogenesis rate [2,3]. Increased expression of angiogenic growth factors has been reported in a murine model of distraction osteogenesis [4,5]. Moreover, blood flow at the distraction site is nearly 10 times that of control sites 2 weeks postoperatively and remains two to three times that of control sites during the consolidation period [6]. Therefore, enhancing angiogenesis during distraction osteogenesis has a potential therapeutic value for new bone formation. Angiogenesis is regulated by various growth factors such as vascular endothelial growth factor (VEGF), angiopoietin, transforming growth factor β, insulin-like growth factor-1, bone morphogenetic protein (BMP), and basic fibroblast growth factor [7]. Among these factors, VEGF has received a great deal of attention owing to its ability to promote angiogenesis and vasculogenesis and has been shown to play a crucial role during osteogenesis [8].

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Angiopoietin 1 (Ang1) is another angiogenic growth factor that generates a stable and functional vasculature via interaction with Tie2 and Tie1 receptors [9,10], which is mainly expressed in vascular endothelial cells [11] and a subset of monocytes/macrophages [12]. Importantly, when coadministered with VEGF, Ang1 can counteract VEGF-induced side effects while continuing to have an additive effect on vessel formation [13,14]. Upon stimulation, Tie2 translocalization in endothelial cell–cell and cell–matrix contacts may be the main molecular event that induces angiogenesis and vascular enlargement [15,16]. The structure of Ang1 consists of a carboxy-terminal fibrinogen-like domain, a central coiled-coil domain, and a short amino-terminal domain [15]. However, large-scale production of Ang1 is hindered by aggregation and insolubility resulting from disulfide-linked higher-order structures. Toward the goal of enhancing the positive effects of Ang1, Cho et al. [15] replaced the amino terminus of Ang1 with the short-coiled coin domain of cartilage oligomeric matrix protein (COMP) to produce a recombinant chimera COMP–Ang1. COMP–Ang1 is more soluble, stable, and potent than naturally occurring Ang1, and long-term treatment with COMP–Ang1 produces a long-lasting and stable vascular enlargement effect as well as increased blood flow [15,16]. COMP–Ang1 was reported to protect against radiation-induced endothelial cell apoptosis [17], promote wound healing in diabetic *db*/*db* mice [18], ameliorate renal fibrosis [19,20], prevent hypertension, enhance insulin sensitivity [21], and protect cerebral ischemia [22]. Recently, we also reported that intraosseously injection of COMP–Ang1 protein effectively repairs osteonecrotic damage and prevents femoral head deformity by promoting angiogenesis and bone remodeling [23]. In the present study, we investigated the possibility of using COMP–Ang1 to facilitate bone lengthening.

Materials and methods

Surgical procedure

Sprague-Dawley rats (350–400 g) were used in these experiments. With the exception of the initial pilot studies, rats were tested in groups of six to nine individuals. The body weights of experimental animals were closely monitored to confirm feeding and expected growth. All rats were handled regularly for at least 1 week before surgery and housed in individual cages at 22 °C and 50% humidity in controlled rooms having 12 h light/dark cycles. Surgeries were performed under semisterile conditions with animals under anesthesia induced by intraperitoneal injection of 100 mg/kg ketamine–HCl and 10 mg/kg xylazine. The surgical site was shaved and prepared with a 70% ethanol solution. Sterile drapes, gloves, and instruments were used. The electric drill was disinfected with alcohol.

The operation was performed on both hind legs in each animal. The fibula was gently broken using mezenbaum scissors through a mini incision. Next, using a monofixator as a template, four (0.9 mm in diameter) transfixing Kirschner wires were drilled into the coronal plane at low speed across the proximal and distal tibiae. Afterward, a lateral monofixator was applied and a 5- to 7-mm longitudinal incision was made over the anteromedial aspect of the tibia. After subperiosteal dissection, a low-energy osteotomy was created between the two middle wires in the proximal diaphysis of the tibia by low-speed oscillating saw. The wound was then closed, and all four transfixing wires were fixed to a pair of monofixators. Postoperative recovery was facilitated in a healing cage with administration of analgesics (utorphanol, 0.1 mg/kg body weight) and antibiotics (topical furazolidone for the tibia). After a 7-day latency period, distraction was initiated at a daily rate of 0.5 mm in two steps for 20 days, followed by a consolidation period of 8 weeks. During the postoperative period, animals were allowed to bear weight on the limbs that had undergone the procedure. Lastly, the animals were anesthetized and sacrificed by lethal injection. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Chonbuk National University.

COMP–Ang1 administration procedure

Recombinant COMP–Ang1 was kindly donated by Dr. GY Koh (KAIST, Daejon, Korea). Rats were divided into three groups: (1) sham group, (2) BSA group, and (3) COMP–Ang1 group. Sham group rats underwent tibial distraction without any treatment. BSA (100 μg) or COMP–Ang1 (100 μg) was injected transcutaneously using a 30-gauge needle into the center of the distraction zone on the last day of distraction.

Radiographic evaluations

Standardized anterior–posterior radiographs of the tibia and fibula were taken immediately after surgery and every 2 weeks postoperatively until sacrifice. We used a mammographic imager with a direct detection flat-panel array design (Mammomat Novation^{DR}; Siemens Medical Solutions; Erlangen, Germany) and a flat-panel digital detector (24 cm × 29 cm; maximum matrix size, 3328 × 4096; pixel size, 70 μm). All images were obtained using exposure settings of 34 kV (peak) and 110 mA s at a magnification of 1.5×.

Assessment of bone formation by nanoCT

A nanoCT (Institute for Radiologic Imaging Science of Wonkwang University, Iksan, Jeonbuk, Korea) was used to evaluate the amount of new bone that formed within defect areas. The unit is intended for the noninvasive imaging of the internal microstructures of objects with submicrometer resolution, and consists of a nanofocus X-ray source, precision object manipulator, and high-resolution CCD detectors. The source of the open-tube type and the minimum focal spot size was <1 μm. The spatial resolution of the nanoCT device could extend down to the submicron level; however, in the present study, we obtained basic transverse images of 3 μm thickness, with the spatial resolution of the nanoCT images ranging from 2 to 30 μm depending on the magnification range. The object manipulator was composed of three linear motion stages for magnification adjustment (X), alignment (Y), positioning (Z), and sample rotation (θ). The X-ray detector contained a straight fiber-optic coupled CCD. The source to detector distance (SDD) was 500 mm, and the magnification range was 1.1 to 25. The region of interest (ROI) was defined as the distance along the diaphyseal axis that spanned the region at which the cortex of the native bone (as seen on a transverse section) discontinued and proximally to the point at which it continued distally. After determining the ROI for each specimen, the contours of the distraction callus were outlined. A global thresholding algorithm was applied at a constant threshold for all specimens. This threshold was chosen as the intensity (gray value) that corresponded to ~45% of the average intensity of the intact cortical bone in the specimens. Voxels with intensities higher than the threshold were considered to contain mineralized tissue. A constrained 3D Gaussian filter (filter width = 0.8, filter support = 1 voxel) was used to partially suppress image noise. Total bone volumes were compared across groups using a Kruskal–Wallis test (ANOVA by ranks) [24]. All aspects of the system were operated using software (Lucion-3D; MeviSYS, Seoul, Korea). Captured images were analyzed using Image J software (release 1.34s; NIH, USA).

Dual-energy X-ray absorptiometry analysis

The bone mineral content of regenerated regions was determined using dual-energy X-ray absorptiometry (DEXA; Lunar PIXImus2, GE, Diegen, Belgium) and an analysis program designed for small animals (PIXImus2 software, GE). Briefly, excised tibiae were scanned using