Human recombinant chimeric protein COMP-Ang1 promotes angiogenesis and bone regeneration in a rat mandible bone defect model

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Statement of Purpose: Angiogenesis plays a critical role in bone development and bone fracture repair. Angiopoietin 1 (Ang1) is an endothelial growth factor essential for embryonic vascular development. Recently, researcher has developed the cartilage oligomeric matrix protein (COMP)-Ang1 by replacing the N-terminal portion of Angiopeotin 1 with the short coiled-coil domain of cartilage oligomeric matrix protein, a chimeric protein of angiopoietein-1 [1]. COMP-Ang1 is known to have more soluble, stable and potent than did the native Ang1 and long term treatment with COMP-Ang1 produces a long-lasting and stable vascular enlargement effect as well as increased blood flow [2]. Previous studies also showed that COMP-Ang1 helps to promote wound healing in diabetic mice [3] and also increases vascularity and osteogenesis in osteonecrotic femoral heads [4]. However, its role in bone regeneration in bone defect model has not been well documented. In this study we hypothesized that the application of recombinant COMP-Ang1 protein helps to enhance the angiogenesis around the rat mandible bone defect and at a glance promotes osteogenesis to regenerate the new bone.

Methods: Eighteen Sprague-Dawley rats were (7-8weeks old) were anaesthetized by intraperitoneal injection of Zoletil (0.4 mL/kg, Virbac Laboratories, Carros, France) mixed with Rompum (10 mg/kg body weight, Bayer Korea Ltd., Korea). Once the animals were anaesthetized completely they were shaved in the location of the mandibular angle where an incision was made in the skin. Plane-by plane muscle dissection and incision of periosteum was performed until the mandible angle was visible and the mandibular posterior border was completely exposed. A lateral complete defect of 4 mm diameter on right sides of the mandibular ramus was created by using a 4 mm trephine bur that was cooled continuously by 0.9% saline solution irrigation. The mandibular defects were divided into 3 groups, and covered by collagen sponge in the following conditions: (1) no treatment as control (n=6), (2) collagen alone (n=6) and (3) collagen sponges soaked with 5 μg of COMP-Ang1 (Enzo Life Sciences, NY, USA) (n=6). Sponges were secured around the mandibular angle and covered the defects completely. The muscle layers were then closed using 5-0 resorbable sutures, followed by closure of the skin layer using 4-0 resorbable sutures. New bone formations at the defect site were analyzed after eight and twelve week healing periods using micro-CT reconstruction, histological and real time RT-PCR analysis.

Results: RNA was isolated from new bone generated in defect area and examined the expression levels of osteogenic genes, including runt-related transcription factor 2 (Runx2) and bone morphogenetic protein-2 (BMP-2). Significantly up-regulated mRNA levels of osteogenenic genes were observed in the COMP-Ang1 group indicating that osteogenesis is triggered by COMP-Ang1. Further, we also assayed expression levels of angiogenic genes such as tyrosine kinase (Tie-2) and platelet endothelial cell adhesion molecule (PECAM-1). COMP-Ang1 also up-regulated the mRNA levels of angiogenic genes. These results suggest that COMP-Ang1 might accelerate new bone formation through promoting angiogenesis. Furthermore, histological evaluation of H&E and Masson's Trichrome stained sections showed that there was no inflammatory reaction around defect area. After 8 and 12 weeks, the central region in the defect space was filled and bridged with new bone in COMP-Ang1 group, whereas only collagen and control group failed to induce new bone formation. Further, mineralized bone and osteocytes formation in COMP-Ang1 group were more compared to others group. This depicts that COMP-Ang1 helps to induced local bone regeneration. Furthermore, to evaluate the osteogenic capacity of COMP-Ang1, the mandible repair in the defect was also analyzed by micro-CT at 8 and 12 weeks. On the COMP-Ang1 group, the emergence of bone islands, ingrowths of new bones and formation of new bone filled the defect area was observed. In sharp contrast, the bone repair was slow and poor in other two groups.

Conclusions: These results suggest that this strategy, using a recombinant protein COMP-Ang1 has a prominent ability to form a new bone by increasing angiogenesis might lead to an improvement in the current clinical treatment of bone defects for periodontal and implant therapy. Thus, this new recombinant protein may use to coat dental and orthopedic materials and could have a significant utilization for the preparation of new biomaterials and may serve as prosthetic materials in patient suffering from bone diseases like osteoporosis and osteonecrosis. However, further study is required to confirm the ability of COMP-Ang1 in diverse animal model and or with different biomaterials.

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