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Expression of vascular endothelial growth factor and its receptors after mandibular distraction osteogenesis

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Abstract. During distraction osteogenesis, angiogenic activity is essential for new bone formation. This study examined the expression of vascular endothelial growth factor (VEGF) and two of its receptors, Flt-1 (VEGFR-1) and Flk-1 (VEGFR-2), in cellular components after mandibular distraction osteogenesis. Unilateral mandibular distraction (0.5 mm twice per day for 10 days) was performed in six mongrel dogs. Two animals each were killed on days 7, 14 and 28 after completion of distraction. The distracted mandibular segments and contralateral undistracted control segments were harvested and processed for immunohistochemical examination. Seven days after distraction, there was a significant increase in the expression levels of VEGF and its receptors in the osteoblasts, osteocytes and immature fibroblast-like cells compared to control specimens. These levels were maintained for 14 days after distraction in the osteoblasts and fibroblast-like cells. Twenty-eight days after distraction, VEGF and VEGFR-1 were expressed only moderately/weakly in the osteoblasts, and no VEGFR-2 expression was detected in the cellular component of the distracted bone. Throughout the observation period, VEGFR-1 expression was stronger than that of VEGFR-2. The expression patterns of VEGF and its receptors suggest that it plays an important role in osteogenesis, and that osteoblasts and immature fibroblast-like cells of the distracted bone may have an autocrine growth effect during distraction osteogenesis.

Key words: distraction osteogenesis; vascular endothelial growth factor; Flt-1; Flk-1; autocrine growth effect.

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Distraction osteogenesis (DO) is a useful method for treating cases demanding the generation of new bone. Despite the fact that DO is used in various fields, there have been few studies[2,7,27,32] undertaken at the cellular and molecular level. These studies suggest that some growth factors, such as transforming growth factor-beta (TGF-β), insulin-like growth factor-I (IGF-I), bone morphogenetic proteins (BMPs) and basic fibroblast growth factor (bFGF), play important roles in new bone formation after DO. In addition, there is increasing interest in the relationship between osteogenesis and angiogenesis during DO[3,6].

Bone formation is closely related to the formation of blood vessels. Several studies[10,11] have shown that osteoblasts and osteoblast-like cells can produce vascular endothelial growth factor (VEGF), and

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that this process may be tightly regulated by osteogenic factors, including TGF-β and IGF-I. It is also the case that angiogenic factors, such as VEGF and bFGF, can amplify osteogenic activity[15]. Of the various angiogenic factors, VEGF is perhaps the most critical driver of vascular formation during angiogenesis and vasculogenesis, and has been demonstrated to play a crucial role during osteogenesis[12].

VEGF is characterized as a heparin-binding angiogenic growth factor that displays a high specificity for endothelial cells, and is structurally related to platelet-derived growth factor[19]. Several VEGF receptors (VEGFRs) belonging to the tyrosine-kinase receptor family have been identified and cloned, e.g. VEGFR-1 (*fms*-like tyrosine kinase receptor 1, Flt-1), VEGFR-2 (fetal liver kinase 1, Flk-1) and VEGFR-3 (Flt-4). VEGFR-2 appears to mediate the differentiation and proliferation of endothelial cells, and the activation of VEGFR-2 by VEGF results in a mitogenic response. VEGFR-1 appears to play an important role in vascular maintenance as well as in the recruitment of endothelial precursor cells during vasculogenesis. The activation of VEGFR-1 by VEGF appears to induce cell migration. VEGFR-3 is believed to play a role in the development of lymphatic as well as blood vessels[19]. It is unclear whether the roles of the VEGFRs in osteogenic cells are similar to those in endothelial cells.

Masood et al.[16] reported the concurrent expression of VEGF and VEGFRs in a number of tumour cells, and suggested that VEGF here functioned as an autocrine growth factor. Several other studies[5,8,17,18] have also demonstrated the simultaneous expressions of VEGF and VEGFRs during osteoblast differentiation. In this study was examined the autocrine growth activity of the cellular components of a distracted bone after mandibular DO, with regard to the expression of VEGF and its receptors.

Materials and methods

Animal model and surgical protocol

Six mongrel dogs, aged between 1 and 2 years and weighing approximately 10 kg, were used in this study. The animal model and surgical protocol were as described previously[21]. All experimentation was performed after gaining authorization from the Animal Center for Medical Experimentation at Gyeongsang National University.

The animals were anaesthetized by an intravenous injection of a mixture of 10 mg/kg of ketamine (Ketalar®, Yuhan Corp., Korea) and 2.0 mg/kg of 2% xylazine (Rompun®, Bayer Korea). The surgical fields were sterilized with betadine solution and 2% lidocaine HCl containing 1:100,000 epinephrine was then injected into the right submandibular skin. After sequential dissection of the submandible, buccal and lingual corticotomies were conducted between the 3rd and 4th premolars, or between the 4th premolar and the 1st molar. The intraoral mandibular distractor (Leibinger, Germany) was then positioned on the buccal cortical bone, after the mandible had been carefully fractured in a linear manner. The distractor rod was exposed by perforating the retromandibular skin. The wound was closed in two layers with 3-0 Vicryl for the platysma and 3-0 nylon for skin. First generated cephalosporin (20 mg/kg; Cefazolin®, Yuhan Corp.) was injected intramuscularly twice a day for 5 days after surgery. After a 5-day latency period, the mandible was distracted for 10 days at a rate of 1.0 mm/day in two increments per day.

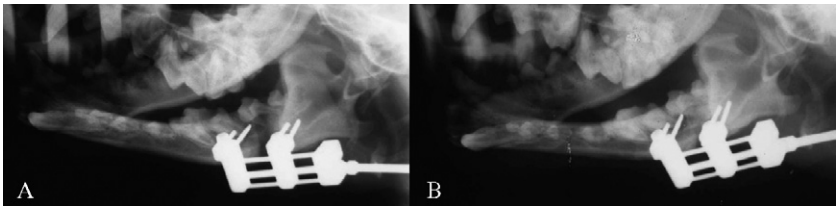

Fig. 1. Radiographs demonstrating the distraction and healing of the mandible. (A) Immediately after completing the distraction, showing a distraction gap. (B) Twenty-eight days after the distraction, note the presence of bone consolidation within the gap.

Specimen preparation

After the administration of general anaesthesia, two animals each were killed by KCl injection at 7, 14 and 28 days after completion of distraction. The right distracted mandibles (the distraction group) and the left undistracted mandible (the control group) were then harvested *en bloc* using an identical procedure. The harvested bony tissue specimens were fixed in 10% neutral buffered formalin for 24 h, and then decalcified in 5% nitric acid for 1 week. After tissue processing,

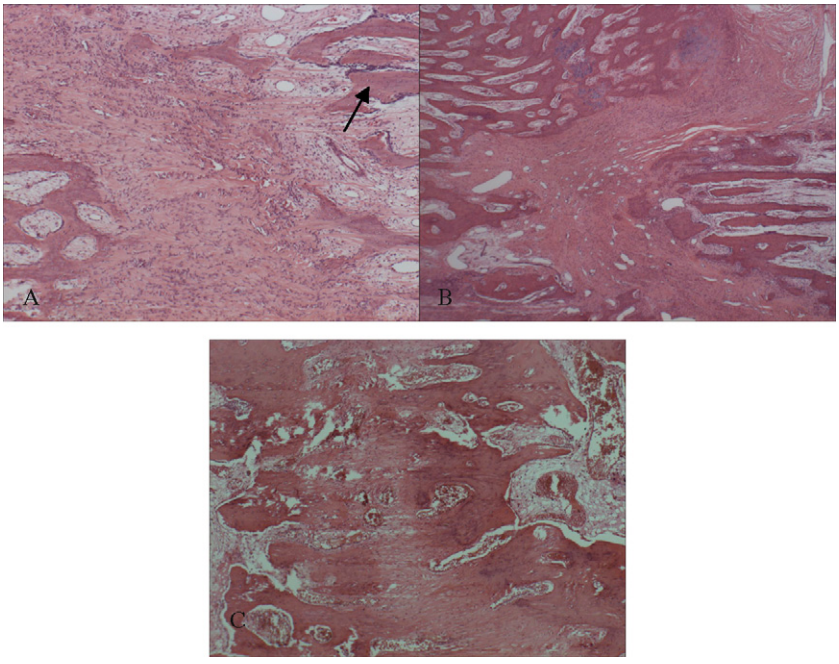

Fig. 2. Hematoxylin and eosin-stained section. (A) Seven days after completing the distraction. Numerous immature fibroblast-like cells were observed in the gap interzone. At this time, many osteoblasts lining the immature trabecular bone were also found (arrow) (original magnification ×40). (B) Fourteen days after the distraction. A fibrous interzone was also present at this time (original magnification ×20). (C) Twenty-eight days after the distraction. The fibrous interzone was almost filled with newly formed bone (original magnification ×40).