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Early healing pattern of statin-induced osteogenesis

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Summary We examined the early histological expressions of vascular endothelial growth factor (VEGF), bone morphogenetic protein (BMP)-2 and core binding factor (Cbfa1) in healing bones with and without a statin (simvastatin). Thirty bone defects were created in the parietal bones of 15 New Zealand white rabbits. In the statin group ($n = 9$), the defects were grafted with carriers of collagen matrix mixed with simvastatin solution, and the animals were killed on days 1 ($n = 1$), 2 ($n = 1$), 3 ($n = 2$), 4 ($n = 2$), 5 ($n = 2$) and 6 ($n = 1$) after operation. In the collagen matrix group, the defects were grafted with carriers of collagen matrix mixed with water for injection, and killed on days 1—6 postoperatively. Immunolocalisation studies of the defects grafted with statin showed that VEGF was expressed on day 3 postoperatively, BMP-2 on day 4, Cbfa1 on day 5 and that new bone was formed by day 5. These events occurred one day earlier than in the group grafted with the carrier alone. The statin induced and accelerated formation of bone locally, and triggered the early expression of growth factors that regulate angiogenesis, differentiation of bone cells, and osteogenesis.

Introduction

The search for an ideal material for bone grafting remains a formidable challenge. Bone morphogenetic proteins (BMPs) are important regulators of osteogenic differentiation during repair of fractures.[1] Wang et al.[2] showed that BMP-2 caused differentiation of a multipotential stem cell line into osteoblast-like cells. To discover small molecules that induce BMP-2, Mundy et al.[3] examined more than 30 000 compounds from a collection of natural products and tested the effects of compounds on the expression of the BMP-2 gene. They identified a statin, a common cholesterol-lowering drug that inhibits hepatic hydroxymethylglutaryl coenzyme A (HMG—CoA) reductase, the rate-limiting enzyme in the meval-

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onate pathway, as the only product in the collection that specifically increased expression of the BMP-2 gene.[3] They also reported that statins stimulated formation of bone in animals.[3]

To examine the possibility that statins could be used clinically in the repair of bony defects, we examined their osteoinductivity with a carrier in rabbits.[4] We showed that 308% more new bone was formed in 14 days in defects grafted with a statin mixed with a carrier of collagen matrix than in those grafted with the carrier alone. Despite its tremendous osteoinductivity, the histological picture and the mechanisms involved in the early healing stage of statin-induced osteogenesis were largely unknown.

Angiogenesis is essential for bony formation. Vascular endothelial growth factor (VEGF) is an angiogenic cytokine. It is a mitogen, a powerful regulator of angiogenesis, and dilates vessels.[5] VEGF has a direct action on endothelial cells.[6] It also stimulates blood vessels to become more permeable and has a central role in the regulation of vasculogenesis. VEGF may also induce proliferation and differentiation of osteoblasts by stimulating endothelial cells to produce osteoanabolic factors.[7] There was a close correlation between expression of VEGF, vascularisation, and formation of bone in the glenoid fossa in rats after forward positioning of the mandible.[8]

The core-binding factor (Cbfa1) gene, also referred to as polyoma enhancer-binding protein (Pebp2αA), runt-related transcription factor-2 (Runx2), and acute myelogenous leukaemia factor (AML-3), is an effector of BMPs and plays a key part in osteoblastic differentiation.[9,10] The role of Cbfa1 in formation of bone has been shown in Cbfa1-deficient mice that have no osteogenesis,[9] and genetic mutations in the Cbfa1 gene are associated with the multiple skeletal abnormalities in patients with cleidocranial dysplasia syndrome.[11] The Cbfa1 protein binds the promoter of several genes that are expressed predominantly in osteoblasts.[9] All the three isoforms of Cbfa1 increase the expression of osteocalcin, osteopontin, and collagen type I genes, and isoforms I and II, but not III, induce expression of alkaline phosphatase (ALP).[12] Forced expression of Cbfa1 in non-osteoblastic mesenchymal stem cells also leads to the acquisition of an osteoblastic phenotype.[9] BMP-2 increases expression of the Cbfa1 type II transcript during osteogenic differentiation of primary rat osteoblasts, MC3T3-E1 osteoprogenitor cells, and C2C12 premyoblasts,[13] and transiently (within an hour) increases the concentration of Cbfa1 mRNA in stromal cells in human marrow.[14] The Cbfa1 mRNA is transiently upregulated by BMP-4/-7, TGF-β1, and ascorbic acid in C3H10T1/2, C2C12, and MC3T3-E1 cells.[9,15]

The purpose of the present study was to examine the early histological expressions of VEGF, BMP-2, and Cbfa1 in healing bony defects with and without a statin using immunolocalisation techniques.

Material and methods

Study groups

Thirty 10 mm × 5 mm full-thickness bony defects were created in the parietal bones of 15 New Zealand white rabbits from an inbred colony. The defects were similar to those in our previous study of a statin.[4] The rabbits were 5 months old (adult stage) and weighed 3.5—4.0 kg. The handling of the animals and the experimental protocol were approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong.

The animals were divided into two study groups. The statin group comprised nine rabbits. Defects were filled with collagen matrix mixed with statin solution, and they were killed on days 1 ($n = 1$), 2 ($n = 1$), 3 ($n = 2$), 4 ($n = 2$), 5 ($n = 2$), and 6 ($n = 1$) after operation.

The collagen matrix group comprised six rabbits, the defects in which were filled with collagen matrix mixed with water. They were killed on days 1, 2, 3, 4, 5, and 6 after operation.

Operations

An hour before the operation the animals were injected with oxytetracycline hydrochloride (200 mg/ml, 30 mg/kg body weight, Tetroxyla, Bimeda, Dublin, Ireland), buprenorphine hydrochloride (0.3 ml/kg body weight, Hypnorm, Janssen Pharmaceutical, Beerse, Belgium), and diazepam (5 mg/ml, 1 mg/kg body weight, Valium 10, Roche, Switzerland). To maintain the extent of neuroleptanalgesia, further doses of Hypnorm (0.1 ml/kg) were given at 30-min intervals during the operation.

The operation comprised the creation of 10 mm × 5 mm full-thickness parietal bony defects (the thickness of the calvarium being roughly 2 mm), devoid of periosteum, guided by templates. In the statin group, the defects were filled with 0.02 g of absorbable collagen matrix sponge (purified bovine fibrillar collagen, Collagen Matrix Inc., NJ, USA), to which was added 0.2 ml statin solution (Zocor® tablet, simvastatin 10 mg, Merck & Co,