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The effect of local simvastatin delivery strategies on mandibular bone formation in vivo

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Abstract

Systemic simvastatin is known to reduce cholesterol and stimulate modest bone formation, but local surgical placement in polylactic acid domes causes robust bone formation and local swelling. A less invasive and more flexible injection protocol was studied to evaluate the bone-inducing effects compared to surgical implantation. Bone formation rate, short- and long-term bone augmentation histology, and mechanical properties were evaluated to characterize the new bone in a rat bilateral mandible model (test and control sides in same animal). Results demonstrated that multiple (3) injections of 0.5 mg simvastatin effectively reduced soft tissue swelling while preserving bone growth (60% increase of bone width at 24 days) compared to simvastatin dome placement (43% increase at 24 days). Compared to controls, bone formation rate was significantly higher on the simvastatin side, especially in the dome. Three-point bending tests revealed higher maximum force to fracture and stiffness at 24 days with simvastatin injections. Long-term evaluation showed that 55% of maximum new bone formed 24 days post-injection was retained at 90 days.

Keywords: Simvastatin; Local delivery; Bone formation; In vivo; Long-term; Mechanical tests

1. Introduction

Inadequate jawbone thickness may lead to subsequent osseous defects, such as fenestration around roots and dental implants. Ridge-augmentation procedures are complex and often unpredictable. Simplified, safe and reliable methods for adding thickness to maxillary/mandibular bone would be an important step in augmentation of the jaw for dental implants or regeneration of periodontal or peri-implant defects. Even minor augmentations of ridge contours require surgical interventions. Previous animal studies have used surgically implanted polypeptide growth factors with graft materials, like bone morphogenetic proteins (BMPs), platelet-derived growth hormone, insulin-like growth factor, and fibroblast growth factor [1–4]. These approaches have resulted in variable bone formation, usually require high doses and expensive grafting composites, and may induce adverse reactions in the host.

Since locally applied statins (specific inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase in the production of cholesterol) [5] were discovered to be potent stimulators of bone formation [6], the possibility of using

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these compounds as practical bone anabolic agents has been postulated. There is evidence that statins promote osteoblast differentiation and enhance BMPs in mouse bone marrow stromal cells [7], and simvastatin increased alkaline phosphatase activity and osteocalcin expression levels in human bone marrow stem cells [8]. In the ovariectomized rat model more new bone formation in mandibular defects and skeletal bone was seen when statins were given orally [9,10], and systemic statin-treated humans showed an increase in bone mineral density, bone-specific alkaline phosphatase activity and osteocalcin after 3 months [11]. However, the effect of statins when administered systemically appears to be minimized by clearance by the liver [12], and systemic side effects of high doses are significant [13].

Local application of statins in healing sites or defects has been shown effective in new bone formation. Statin/collagen matrix grafts applied to the rabbit's calvaria caused expression of BMP-2, vascular endothelial growth factor and core binding factor 1 in healing bone within 5 days, and 308% more bone than collagen matrix controls [14–16]. We previously have shown that surgical application of low dose (0.5 mg) simvastatin (semisynthetic 2,2-dimethy butyrate analogue of lovastatin) in methylcellulose gel under polylactic acid dome membranes implanted in a bilateral rat mandible model was effective in inducing new bone comparable to >1.0 mg doses, but doses ≤ 1.0 mg caused less surrounding inflammation than the higher doses [17]. However, some soft tissue swelling occurred even in the control gel/dome membrane side, presumably due to the surgical trauma. For a more flexible approach to augment jaw width, we hypothesized that drug delivery by multiple injections should allow efficient applications to initiate and augment oral bone growth accompanied by minimal tissue damage and inflammation. Furthermore, regenerated bone should provide acceptable mechanical properties and function as normal bone, with significant amounts retained over extended periods of time. Our studies are based on multiple injections of simvastatin to enhance new bone formation in rat mandibles added onto existing bone in vivo. We used low dose simvastatin suspended in methylcellulose gel without combining any bone graft or scaffold materials. We evaluated and characterized bone-inducing capacity, histologic cellular and tissue populations, bone formation rate and mechanical properties comparing surgical dome implants and single and multiple injections. We also investigated histological bony change and mechanical properties after 90 days to explore long-term stability of new bone.

2. Materials and methods

2.1. Animal procedures

All animal procedures were approved by the Institutional Animal Care & Use Committee at the University of Nebraska in accordance with National Institutes of Health Guidelines. A bilateral mandible model using mature female Sprague Dawley rats (Harlan Teklad, Madison, WI) was used for these experiments. The rats were weighed at the day of simvastatin application and again the day of euthanasia. Prior to applying simvastatin all rats were sedated by intraperitoneal injection of 2 parts ketamine (100 mg/ml) and 1 part xylazine (20 μl/ml) at a dosage of 0.1 ml/100 g (Phoenix Pharmaceutical, St. Joseph, MO) supplemented with local 0.2 ml 3% mepivacaine without vasoconstrictor (Cook Waite, Chicago, IL) as necessary.

Simvastatin of 0.1, 0.5, or 1.0 mg in 30 μl methylcellulose gel under a polylactic acid dome membrane (SIM-DOME) was surgically implanted supraperiosteally on one side of the mandible, and gel alone in dome membrane (GEL-DOME) was applied on the other, as described previously (Fig. 1) [17]. The previous study showed that the 0.5 mg dose in domes produced the best bone growth/inflammation ratio. Therefore, a single injection of 0.5 mg simvastatin, or 3 weekly injections of 0.1 mg (suboptimal dose for DOME) or 0.5 mg simvastatin in 50 μl methylcellulose gel (SIM-INJ) was applied to separate rats with gel alone (GEL-INJ) on the contralateral side (Fig. 1). These experimental conditions are outlined in Table 1. Active drug treatments were randomized to right and left sides, and placed over the lateral aspect near the inferior angle of the mandible. Injections were done using a 20 gauge and 1 cm length needle at a marked mandible angle 6 mm deep into the tissue. Muscle was penetrated and a space was created supraperiosteally by moving the needle side-to-side at the same location used in other animals for dome implants. After healing times (3,

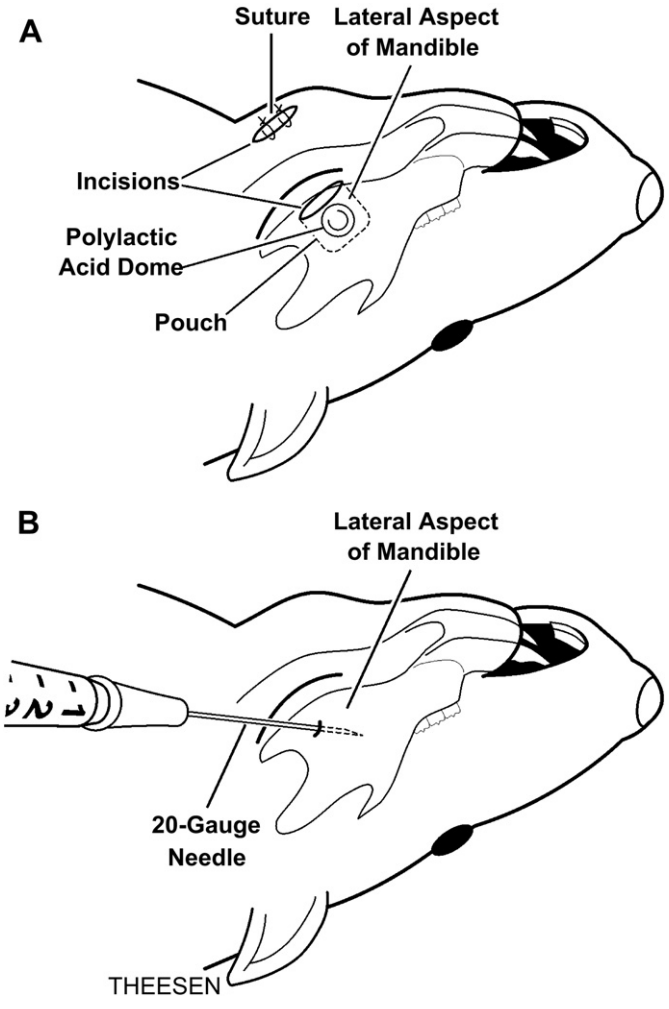

Fig. 1. Diagram of simvastatin application to rat mandible. (A) Polylactic acid domes carrying simvastatin (SIM-DOME) in methylcellulose gel or gel alone (GEL-DOME) were inserted through an incision into the pouch (dotted line) made on lateral aspect of the mandible, then closed with suture. (B) Injections of simvastatin in 50 μl methylcellulose gel (SIM-INJ) or gel alone (GEL-INJ) were applied using a 20 gauge and 1 cm length needle in a marked mandible angle 6 mm deep into the tissue.