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Effects of local simvastatin on periosteal distraction osteogenesis in rabbits☆

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Abstract

Our aim was to evaluate the effect of local simvastatin on the formation of new bone using a new design of periosteal distractor. The distractors were placed between the periosteum and bone at the inferior border of the mandible of 20 New Zealand rabbits. In the first group ($n = 10$) simvastatin was applied locally to the distraction zone. The other 10 rabbits served as controls. The formation of new bone was evaluated with digital direct radiography, computed tomography (CT), and histomorphometric analyses. New bone formed in all rabbits, but more formed in the experimental group according to histomorphometric variables. However, other measurements did not differ significantly between the groups. The new design of the periosteal distraction device was successful in causing new bone to form. Local simvastatin made no significant contribution to the procedure.

Keywords: Periosteal distraction; Simvastatin; Bone augmentation; Osteogenesis

Introduction

The "gold standard" for the reconstruction of bony defects remains autogenous grafting,[1] but morbidity at the donor site and the limited quantity of bone available are the main disadvantages.[2–7] Distraction osteogenesis has the potential to overcome these disadvantages, and in addition can provide lengthening of soft tissue together with new bone.[5–7] Various techniques of distraction osteogenesis have been described with reasonable success rates, and in recent years the idea of osteogenesis by periosteal distraction for the treatment of bone deficiencies has also been suggested.[1,2,8,9]

The highly vascularised internal osteoblastic layer of periosteum plays a part in distraction osteogenesis. It is composed of mesencymal stem cells,[5] and it has therefore been suggested that it is more important than endosteum in distraction osteogenesis.[9,10]

Statins are effective lipid-lowering drugs that are widely used to reduce the risk of cardiovascular disease given their ability to inhibit the 3-hydroxy-3-methylglutaryl-coenzyme.[11] They are extensively bound to plasma proteins and predominantly metabolised by the cytochrome P450 family of enzymes, and their main route of elimination is through bile after being metabolised by the liver.[12] Several studies have shown that both systemic and local simvastatin contribute to bony regeneration.[11–15] Although the exact mechanism is not known some hypotheses have been

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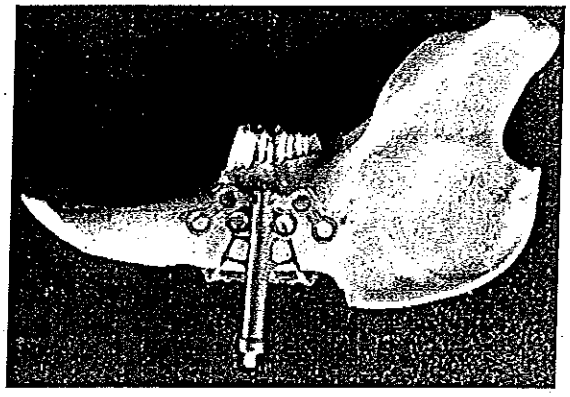

Fig. 1. Photograph of the orientation of the device to the rabbit mandible; lateral view.

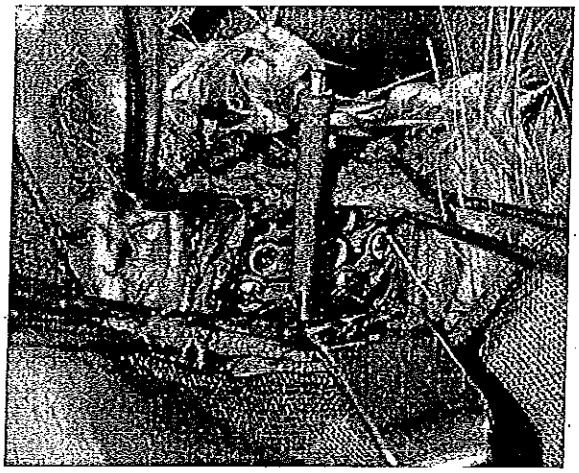

Fig. 2. Intraoperative photographs showing fixation of the device with screws laterally.

proposed, which are mostly based on the increased expression of the bone morphogenetic protein-2 (BMP-2) gene in bony cells after statins have been given.[12–15] It has also been shown that statins (including simvastatin) stimulate the expression of vascular endothelial growth factor in osteoblasts through reduced protein prenylation and the phosphatidylinositide-3 kinase pathway, which promotes osteoblastic differentiation.[16]

In this study we have evaluated the effects of local simvastatin on the formation of new bone through a periosteal distractor that was designed and manufactured particularly for this study in rabbits' mandibles.

Material and methods

The study was reviewed and approved by the ethics review committee of Cukurova University Medical Scientific Research Centre. Twenty skeletally mature New Zealand rabbits weighing 2.8–3.2 kg (mean (SD) 3.05 (0.15) kg) were divided into two groups of 10 each. The same periosteal distractor and distraction procedure were used in both. Simvastatin-soaked gelatin sponges were placed on the distraction zone during the operation in the study group, and dry gelatin sponges were used in the control group.

A new periosteal distraction device was designed and manufactured for this study (Synthes, Solothurn, Switzerland). The distraction device is made of pure titanium and there are three components; mesh (the easily bendable part of the device), the distractor, and the fixation plate. It was designed to be adjustable to the inferior border of the rabbit mandible. It raises the periosteum and other soft tissue layers in a superior direction, and allows a distraction gap to be created between the periosteum and the cortex (Fig. 1).

Surgical technique

After the general anesthesia had been maintained with IM 35 mg/kg ketamine (Alfamyne, Egevet, Izmir, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Istanbul, Turkey) the animal was placed supine and the distractors put in place. A skin incision about 2 cm long was made parallel to the inferolateral surface of the mandible. Care was taken to make the incision as far as possible from the distraction site (1–2 cm lateral to the inferior border) to keep the periosteum intact. The dissection continued through the subcutaneous and muscle layers. The periosteum was carefully raised to expose the inferior border of the mandibular bone. When the surface of the bone was exposed, the cortex was perforated with a thin, round bur. The distractor was fixed to the lateral aspect of the mandible with 4 titanium screws (Fig. 2). Before the distractor was finally placed, the adjustment was checked, the gelatin sponge was inserted, and the wound closed.

In all animals, a gelatin sponge was placed between the mesh and the surface of the cortical bones. In the study group, the sponge was soaked with simvastatin, while in the control group it was not. Commercially available simvastatin tablets (Zocor 10 mg, Merck Sharp & Dohme, Istanbul, Turkey) were used. A concentration of simvastatin of 2.5 mg/ml was prepared by dissolving a quarter of each tablet in saline. The dose of simvastatin was the same as that used in a previous study in rabbits.[15] The solution was soaked in 0.02 g of gelatin sponge. After the distractor had been secured in its final position it was filled with gelatin sponges. The soft tissue layers were sutured primarily with 4/0 resorbable sutures (Vicryl, Ethicon, Brussels, Belgium).

Postoperative care

Tramadol 1 mg/kg and cefazolin 25 mg/kg were given intramuscularly preoperatively and twice a day for 4 days postoperatively. The rabbits were housed in separate cages and fed soft food for a week. After the first week, normal

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Table 1
Comparison of mean (SD) values in Hounsfield units (HU) according to computed tomographic assessments ($n = 10$ in each group).

	Simvastatin group	Control group
Density at distraction gap	378.7 (138.2)	235.0 (120.5)
Density at pristine bone	717.0 (202.0)	566.9 (92.2)
Percentage	52.6 (14.7)*	40.1 (18.0)*

* $p = 0.14$.

diet was resumed. Intake of food and water and weight were monitored and recorded daily.

Distraction procedure

After a 7-day latency period the distractors were activated by 0.35 mm/day for 10 days (3.5 mm in total). The consolidation phase lasted 45 days, and after it had been completed the animals were killed with an intravenous injection of sodium pentobarbitone 100 mg/kg.

Computed tomography

After the mandibles had been harvested they were scanned by computed tomography (CT) (ILUMA CT, Ardmore, OK, USA), and densitometric evaluations made with CT software. The mean (SD) value of the densitometry measurements of the newly-formed bone at the distraction gap and the pristine bony area were defined on the same slices according to the CT software. The densitometric values were expressed in Hounsfield Units (HU). For statistical analysis, the ratio of the densitometric value of the newly-formed bone to the pristine bone was calculated for each specimen.

Direct digital radiography

After the CT evaluations, sagittal sections 3 mm thick were prepared using an electric diamond saw and grinding system (Exakt, Norderstedt, Germany). Digital radiographs of the slices of the distracted areas were obtained with an aluminium step wedge attached to the sensor of the digital radiographic device (RVG, Trophy Radiologie, Vincennes, France). The aluminium step wedge consisted of 10 steps, the thickness of which ranged from 0.5 to 7 mm. The same aluminium step wedge was used for all radiographs. The density of the bone was measured on digital images using image analysing software (Image J 1.23J, Bethesda, MD). The grey level of each step of the aluminium step wedge was measured and used for calibration of the software. The aluminium equivalent of bone density was calculated for the distraction gap and the pristine bone.

Histomorphometric evaluation

After the direct digital radiography assessments, the specimens were further prepared for histomorphometric analysis using the electric diamond saw and grinding system until they were 100 μm thick, and then they were stained with toluidine blue. Digital images of the sections were analysed by histomorphometric software (TAS V1.2.9; West Yorkshire, UK). Bone volume, bone surface, and trabecular thickness were calculated for each specimen. The nomenclature and calculations for bone histomorphometry were used in accordance with the report of the American Society for Bone and Mineral Research.[17]

Table 2
Mean (SD) bone density (mm aluminium) according to direct digital radiographic images ($n = 10$ in each group).

	Simvastatin group	Control group
Density at distraction gap	0.74 (0.13)	0.69 (0.13)
Density at pristine bone	0.95 (0.11)	0.93 (0.10)
Percentage	77.8 (6.8)*	74.1 (6.5)*

The aluminium step wedge consisted of 10 steps, the thickness of which ranged from 0.5 to 7 mm. The same aluminium step wedge was used for all radiographs. The density of the bone was measured on digital images using image analysing software (Image J 1.23J, Bethesda, MD). The grey level of each step of the aluminium step wedge was measured and used for calibration of the software. The aluminium equivalent of bone density was calculated for the distraction gap and the pristine bone.

* $p = 0.12$.

Statistical analysis

The sample size of the study was based on a power analysis that considered important mean difference in bone volume between the groups as 10%, SD 5%, probability of the difference having arisen by chance 0.05, and power 0.8.[9] The significance of differences between histomorphometric and radiodensitometric tests were assessed using the Mann–Witney *U* test. Probabilities of less than 0.05 were accepted as significant.

Results

All animals tolerated the procedures well and survived until the end of the study. None of them had any noticeable weight loss or other local or systemic complications. The distraction was successfully executed in all rabbits. Gross examination of the harvested mandibles showed that all distractors were stable and were well incorporated into the neighbouring tissues.

The mean (SD) ratios of the HU values of mineralised tissue at the distraction gap to the pristine bone in the control and study group were 40.1 (18.0)% and 52.6 (14.7)% respectively (Table 1). There was no significant difference between the 2 groups ($p = 0.14$) (Table 1).

The mean (SD) ratios of the aluminium equivalent of the thickness of mineralised tissue at the distraction gap to pristine bone were 77.8 (6.8)% for the experimental group and 74.1 (6.5)% for the control group (Table 2 and Fig. 3), but the difference was not significant ($p = 0.19$).

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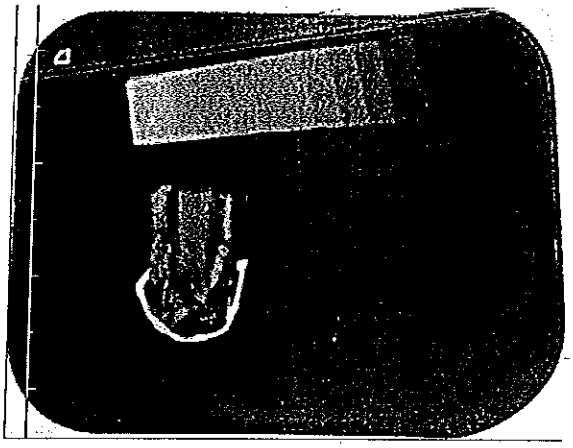

Fig. 3. Digital radiographic image of a specimen from the control group. Note the presence of newly-formed bone between the bone and the mesh in both specimens.

Table 3
Mean (SD) histomorphometric variables ($n = 10$ in each group).

	Simvastatin group	Control group
Bone volume (%)	66.0 (5.7)*	57.5 (6.5)*
Bone surface (mm/mm^2)	34.6 (7.4)	36.0 (9.8)
Trabecular thickness (μm)	0.27 (0.05)	0.32 (0.09)

* $p = 0.01$.

On histological examination the original bone trabeculae were oriented parallel to the distraction vector. The newly-formed bone tissue and connective tissue were present under the titanium mesh in all specimens. Woven and lamellar bone had relatively thick trabeculae, and the newly-formed bone was characterised by both an increase in the number of osteocytes/unit area and an increase in trabeculae. This result was similar in both groups, but the simvastatin group had slightly thinner trabeculae. The mean bone volume (total amount of new bone) was significantly higher in the study group than the control group ($p = 0.02$). The mean bone surface area and trabecular thickness did not differ significantly between the groups. (Table 3).

Discussion

We have assessed the effect of a new periosteal distractor together with local simvastatin on the regeneration of bone. The technique is based on the principle that gradual distraction of the periosteum from the bone results in the formation of new bone.[11] We used a new distractor that was designed and developed by our group. We are the first group to our knowledge that has studied the contribution of simvastatin to periosteal distraction-aided bony regeneration in vivo.

Although the bone-forming capacity of the periosteum has been known for longer than a century, bony regeneration using a periosteal distractor has been studied only during the past decade. Only animal studies have been published, to our knowledge, and different devices have been proposed.[1,2,8,9,18] Previously-described distractors required incisions on the periosteum to adapt and secure the device in position or to allow a distraction rod to perforate the flap. However, damage to the periosteum impairs the distraction,[8,18] so it is important to keep the periosteum intact while the device is being inserted. The distractor rod is located on the lateral aspect, so the rod perforates the periosteum and other soft tissue layers from the lateral aspect of the distraction zone. It is possible therefore to make the incision far from the distraction zone and preserve the continuity and integrity of the periosteum over the distraction zone. We think that the successful regeneration of bone that we achieved is mainly because of this.

A previous study by Oda et al. showed that decortication of the bony cortex improves the quantity of newly-generated bone from periosteal distraction osteogenesis.[9] We also decorticated the bony surface before the device was inserted, and placed a gelatin sponge between the mesh and the cortical bone of the mandible in every case, the main purpose of which was to deliver simvastatin to the region. However, this may also improve healing by stabilising the blood clot that accumulates in the area.

Several previous studies have shown accelerated healing after the topical application of simvastatin,[19–21] and its effects on bony healing have been shown in bony defects, extraction sockets, and healing fractures. In the present study, radiological and histomorphometric variables showed slightly but not significantly better results in the animals given simvastatin. In such conditions, success of the topical use of a medium depends on various factors, including the type of carrier, type of scaffold, concentration of the medium, and presence of a controlled delayed delivery.

We used gelatin sponge as a carrier and a simvastatin dose of 2.5 mg/ml in a single dose at the time of placement of the distractors. The elution of the drug over the following days was not adjusted, and the plasma concentration of simvastatin was not measured. As the gelatin sponge had dissolved completely by the end of the consolidation period, the concentrations of simvastatin at one week are not known, and so a dose response curve cannot be calculated. Future studies using different methods of delivery and different concentrations of the drug may give different results.

The quantity and the morphology of the newly-formed bone were assessed by direct radiography, CT, and histomorphometric examination. All examinations provided numerical data that we were able to compare objectively, and the results showed that although the experimental group gave slightly favourable results, none of the variables differed significantly between groups except bony volume. In previous studies reported distraction rates have varied from 0.2 to 0.5 mm/day,[22–24] but we have shown that a distraction rate of 0.35 mm/day gave reasonable results.

According to the histomorphometric assessment, the mean bone volume was significantly higher in the study group than in the control group. However, the specimens in the

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study group had slightly thinner trabeculae than those in the control group. Bone volume was defined as the ratio of mineralised and unmineralised bone volume: total volume of tissue estimated from the analysed specimens.[17] Our results indicate that the application of simvastatin to the distraction gap resulted in increased bone volume, which is not necessarily composed of mineralised and well-organised bony trabeculae.

In conclusion, periosteal distraction osteogenesis is a viable method for reconstruction of resorbed alveolar ridges. The design of the periosteal distraction device used in this study allowed its placement without damage to the periosteum. The inferior border of the rabbit mandible is a feasible model for periosteal distraction studies, but it was not possible to find out whether there was any simvastatin present at the site of distraction, so there is no strong evidence of the contribution of local simvastatin to bony regeneration with this periosteal distraction technique.

Conflict of interest

We have no conflict of interest.

Ethics statement

The study was approved by the Ethics Review Committee of Cukurova University, conducted in accordance with the guidelines of the Cukurova University Animal Research Centre, and supported by Cukurova University Scientific Research Projects Grant, Adana, Turkey (DHF2012D08).

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