

Removal Torque Analysis of Implants in Rabbit Tibia After Topical Application of Simvastatin Gel

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The aim of this study was to evaluate the effects of topical application of simvastatin gel (7.5 mg) on the removal torque of titanium implants in the rabbit tibia. A total of 32 surgeries were performed on 16 New Zealand rabbits for the placement of 2 implants in 1 tibia of each rabbit. Only 1 of the surgical defects was injected with 30 mg/mL of simvastatin gel before implant placement. The initial torque was set at 20 N.cm, and removal torque testing was performed 28 and 56 days postoperatively with a Tonishi torque wrench. Surgical defects were divided into 4 groups: group IG-28 (test, 28 days), group IG-56 (test, 56 days), group I-28 (control, 28 days), and group I-56 (control, 56 days). Removal torque values were higher in group IG-56 than in groups IG-28, I-28, and I-56 ($P < .05$). Groups IG-28, I-28, and I-56 showed similar values ($P > .05$). Removal torque force increased under the influence of simvastatin, indicating that topical administration of a 7.5-mg dose of simvastatin gel is effective in improving the torque force required to remove implants inserted in the rabbit tibia.

Key Words: *statin, removal torque, dental implants*

INTRODUCTION

Advances in research on the treatment of hyperlipidemia in the mid-1980s culminated in the development of a revolutionary class of drugs: the statins or vastatins. These lipid-lowering drugs can reduce low-density lipoprotein levels by 29%, reaching a 60% reduction in cholesterol levels in some cases.¹⁻³ All statins share the ability to inhibit endogenous cholesterol synthesis by competitively inhibiting 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thus preventing the conversion of HMG-CoA reductase to mevalonate. On the other hand, statins can increase bone mineral density, reducing the risk of osteoporosis and fracture.⁴⁻⁶

Topical statins alone and/or associated with a vehicle that slows their intestinal absorption and hepatic metabolism, such as gel, stimulate bone formation by acting on the mRNA synthesis of bone morphogenetic protein-2 (BMP-2), which promotes osteoblast proliferation and differentiation. Since this mechanism precedes the increase in BMP-2 mRNA expression, statins may also promote angiogenesis.⁴⁻⁶

Bone morphogenetic protein-2, a member of the transforming growth factor- β family, may have direct effects on adjacent cells (paracrine effect) and/or act on osteoblasts (autocrine effect), thus aiding bone regeneration over a long period of time. This protein may progress into a component of bone remodeling over a long time, thus enhancing the pattern of bone mineral density.⁷

Statins have also been demonstrated to induce osteoclast apoptosis and to inhibit bone resorption *in vitro*; after *in vivo* treatment, they promote a decrease in osteoclast numbers.^{8,9} It is therefore

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crucial to understand the effects of topical statin gel on surfaces and surgical defects in order to increase the success rate of implant osseointegration in patients with osteoporosis or other hypocalcemic disorders.^{10,11}

Complementary simvastatin partially prevented the ovariectomized-induced bone loss in 8-month-old rats.³ Using a healing femur fracture model, simvastatin given orally in the diet resulted in a marked increase in callus amount, fracture-breaking strength, and energy absorption at day.¹²

In addition, local application seems reasonable as currently available statins were developed to target liver metabolism. These statins are poorly distributed to bone, and very few of an oral dose reach the systemic circulation. Furthermore, local application avoids systemic side effects.¹³

The aim of this study was to evaluate the effects of topical application of 30 mg/mL of simvastatin gel (7.5 mg) on the removal torque force of implants installed in the rabbit tibia.

METHODS

Sixteen male New Zealand rabbits, weighing between 3.0 and 3.4 kg and aged 11 to 15 months, were used in this study. The study was approved by the Research Ethics Committee of Santo Amaro University (UNISA), Brazil (protocol No. 133/05). All animals were bred in the animal facilities of Universidade Paulista and, during the experimental period, were individually housed in iron cages, under the same environmental conditions, and fed standard chow and water ad libitum. Rabbits were weighed before surgery to establish the optimal postoperative drug dose (0.6 mL of ketamine hydrochloride, 0.3 mL of midazolam, and 0.5 mL of meperidine intramuscularly) and anesthetic induction (1.0 mL of intramuscular ketamine, injected 10 minutes after preanesthetic medication; anesthetic maintenance consisted of 0.4 mL of ketamine every 15 minutes until the end of surgery). After sedation, trichotomy and preoperative skin antisepsis were performed. Prolacaine hydrochloride and felypressin (0.03 IU/mL) were used for infiltration anesthesia in the tibial region.

Surgical technique

A skin incision approximately 5 cm long was made in the anterior tibia. After incision of the muscle

fascia, the periosteum was stripped using a Molt curette, leading to exposure of the underlying bone. A 16:1 contra-angle handpiece coupled to a surgical micromotor was previously set for 1700 rpm, and the peristaltic pump was set for maximum flow. Two drill holes were made at least 150 mm apart in the right tibia of rabbits to receive 2 implants. The first hole was initially drilled with a gun drill (2 mm) on the medial aspect of the tibia near the knee joint, avoiding distal cortical perforation. The second hole was made using the same drill, the farthest possible from the first hole, toward the paw joint. Distal cortical perforation was avoided to reduce fracture risk. A helicoidal drill (2.8 mm) was used subsequently. A threaded male fastener, with the same diameter of the implant, was then used to facilitate implant insertion and to uniformly control placement torque force, standardized at 20 N.cm.

Implant placement

Implants were inserted using a Torque-Lock torque wrench. Each animal received 2 titanium implants (LTX, 3i Innovation; 3.25 × 8.5 mm, with surface treatment: blasting with aluminum oxide and acid attack) in the right tibia. Before implant placement, 0.25 mL simvastatin gel (30 mg/mL), at a dose of 7.5 mg, was injected in only 1 of the surgical defects (group test: IG). Before statin injections, a sterile gauze was applied to the surgical defect to absorb and stop bleeding, ensuring that the drug could spread along the wall of surgical defects. Only blood was left within the second defect before implant placement (group control: I). After implant placement, running sutures were performed for the inner layer and simple skin suture for the outer layer. Occlusive dressings were then applied to the wounds. Animals were killed after 28 and 56 days, when a new intervention was performed for exposure and removal of implants. A calibrated Tonishi torque wrench was used to record the torque force required to remove implants.

Surgical defects were divided into 4 experimental groups, according to time of reopening and application of simvastatin gel to the surgical site, as follows:

- Group IG-28 (test, 28 days): Eight implants were installed following simvastatin gel injection (30 mg/mL); the sites were reopened 28 days after implant placement for removal torque testing.

TABLE 1

Results from removal torque analysis* of implants in the rabbit tibia in groups IG-28 (test, 28 days, topical simvastatin gel), I-28 (control, 28 days, without topical simvastatin gel), IG-56 (test, 56 days, topical simvastatin gel), I-56 (control, 56 days without simvastatin gel)

Animal No.	IG-28	I-28	IG-56	I-56
1	16	49		
2	29	23		
3	33.5	28.5		
4	43	21		
5	36	24.5		
6	34	22		
7	27	25		
8	31.5	30.5		
Mean \pm SD	31.25 \pm 7.83	27.94 \pm 9.09		
9			48.5	29
10			39	33
11			60	28
12			60	54
13			47	25
14			50	32
15			41	27
16			46	27
Mean \pm SD			48.93 \pm 7.74	31.87 \pm 9.32

*Values are expressed as N.cm.

- Group IG-56 (test, 56 days): Eight implants were installed following simvastatin gel injection (30 mg/mL); the sites were reopened 56 days after implant placement for removal torque testing.
- Group I-28 (control, 28 days): Eight implants were installed without topical simvastatin gel; the sites were reopened 28 days after implant placement for removal torque testing.
- Group I-56 (control, 56 days): Eight implants were installed without topical simvastatin gel; the sites were reopened 56 days after implant placement for removal torque testing.

After the animals were killed, their tibiae were immediately positioned and fixed in a splint to prevent any movement. Removal torque testing was then performed on all implants. An engaging device was used to maintain the torque wrench aligned along the axis of the implant.

Statistical analysis of removal torque values was performed using a Student *t* test for 2 paired samples, with a significance level of .05.

RESULTS

Results from removal torque analysis of implants in the rabbit tibia performed 28 and 56 days after surgery are described in Table 1. Table 2 shows the

analysis of differences found between groups IG-28 (test, 28 days) and I-28 (control, 28 days), between groups IG-56 (test, 56 days) and I-56 (control, 56 days), and between groups IG-56 (test, 56 days) and IG-28 (test, 28 days), using a Student *t* test (2 paired samples for mean values). Table 3 describes the differences found between IG-56 and I-28, I-56 and IG-28, and I-56 and I-28B using a Student *t* test (2 paired samples for mean values). Table 4 shows the results of analysis of variance, 2 criteria.

Significant differences were observed in mean values between group IG-56 (test, 56 days) and group I-56 (control, 56 days; $P = .04$; Table 2). Removal torque values were higher in group IG-56 (test, 56 days) than in group I-56 (control, 56 days; Tables 1 and 2). Mean values for group IG-56 were not higher because of a discrepancy observed in the results for animal 16 (Table 1).

There were no statistically significant differences in mean values between group IG-28 (test, 28 days) and group I-28 (control, 28 days; $P = .448$; Table 2). Removal torque force was evidently higher in group IG-28 (test, 28 days) than in group I-28 (control, 28 days); however, the statistical analysis revealed no significant differences because of a discrepancy observed in the results for animal 1 (Table 1).

Significant differences were observed in mean values between group IG-28 (test, 28 days) and

TABLE 2

Removal torque analysis* 28 and 56 days after surgery between groups IG-28 (topical simvastatin, 28 days) and I-28 (without topical simvastatin, 28 days), groups IG-56 (topical simvastatin, 56 days) and I-56 (without simvastatin, 56 days) and groups IG-28 (topical simvastatin, 28 days) and IG-56 (topical simvastatin, 56 days)

Groups	28-Day Comparison		56-Day Comparison		Test Group Comparison	
	IG-28	I-28	IG-56	I-56	IG-28	IG-56
Days of surgery	28	28	56	56	28	56
Mean	31.25	27.94	46.6	34.3	31.3	46.6
Variance	61.29	82.67	121.1	105.6	61.3	121.1
Standard deviation	7.83	9.09	11.0	10.3	7.8	11.0
Observations	8	8	8	8	8	8
Start <i>t</i>	0.781		-2.31		3.21	
<i>P</i> value	.448		.04		.003	
Two-tailed critical <i>t</i>	2.145		2.14		2.14	

*Student *t* test (2 samples assuming equivalent variance).

group IG-56 (test, 56 days; *P* = .003; Table 2). Our analysis revealed statistically significant differences in mean values between group IG-56 (test, 56 days) and group I-28 (control, 28 days; *P* = .002; Table 3). However, there were no significant differences in mean values between group IG-28 (test, 28 days) and group I-56 (control, 56 days; *P* = .21; Table 3).

DISCUSSION

The present study evaluated the effects of topical simvastatin gel on the removal torque force of implants inserted in the rabbit tibia.

Several studies have associated statins with increased BMP-2 gene expression in bone cells.^{3,6,10-14} This motivated us to study the role of statins in implant osseointegration in the rabbit tibia.

Studies show that statins act by stimulating the formation of BMP-2,³⁻¹⁴ by promoting osteoblast differentiation and bone formation,^{4,15} and by encouraging angiogenesis.^{3,12,15} Statins have also been associated with increased expression of BMP-2 gene in bone cells.^{16,17}

The time of sacrifice adopted in this study was based on studies by Marx and Garg,¹⁷ who showed that the remodeling process of the bone-implant interface in rabbits lasts 6 weeks. Those authors also observed that the maturation of compact bone occurs in 18 months.

Authors assessing the effect of statins when administered systemically or orally have not reported satisfactory results.^{15,18} Therefore, the present study used topical simvastatin gel, injected into the surgical defect before implant placement.

Topical statins alone and/or associated with a

TABLE 3

Removal torque analysis* 28 and 56 days after surgery between groups IG-28 (topical simvastatin, 28 days) and I-56 (without simvastatin, 56 days), groups IG-56 (topical simvastatin, 56 days) and I-28 (without simvastatin, 28 days), and groups I-56 (without simvastatin, 56 days) and I-28 (without simvastatin, 28 days)

Groups	Test 28 Days and Control 56-Day Comparison		Test 56 Days and Control 28-Day Comparison		Control Group Comparison	
	IG-28	I-56	IG-56	I-28	I-28	I-56
Days of surgery	28	56	56	28	28	56
Mean	31.3	34.3	46.6	27.94	27.9	34.3
Variance	61.3	105.6	121.1	82.67	82.7	105.6
Standard deviation	7.8	10.3	11.0	9.09	9.09	10.28
Observations	8	8	8	8	8	8
Stat <i>t</i>	0.66		3.69		1.30	
<i>P</i> value	.21		.002		.21	
Two-tailed critical <i>t</i>	2.14		2.14		2.14	

*Student *t* test (2 samples assuming equivalent variance).

TABLE 4

Analysis of variance results

	GL	SQ	QM	F	P
Treatments	3	2143.68	714.56	10.25	.0004
Blocks	7	572.87	81.83	1.75	.3586
Error	21	1463.93	69.71		

vehicle that slows their intestinal absorption and hepatic metabolism can promote bone formation.^{4-6,19} In the present study, statin gel (7.5 mg simvastatin) had a positive effect on the removal torque force of implants inserted in the rabbit tibia.

Group IG-56 (test, 56 days), when compared with the other groups, showed the highest mean removal torque force, suggesting that the action of statins is enhanced over time (Table 3). Although a comparison between mean values from groups IG-28 (test, 28 days) and I-28 (control, 28 days) showed no statistically significant differences (Table 2), there was a discrepancy in the results for animal 1, in which removal torque force in group I-28 (control, 28 days) was significantly greater than in group IG-28 (test, 28 days; Table 1). This fact could lead us to assume that in group IG-28, statin had no positive effect on removal torque force. However, overall results showed that statin increased removal torque force in all other animals at 28 days. The increase observed in removal torque force in group IG-28 (test, 28 days) may be interpreted as an improvement in the bone-implant interface (ie, we may assume that there was an improvement in bone mineral density). This suspicion may be confirmed by histological analysis of the areas of interest. The absence of such analysis may be considered a limitation of this study; therefore, studies using this approach are warranted to clarify the effects of statin. On the other hand, our results can and should be elucidated by understanding the mechanism by which statins perform their effects on bone and endothelial cells, a mechanism of action that has been demonstrated by several authors assessing statins as modulators of bone formation.^{4-6,19}

The simvastatin dose (7.5 mg) used in this study was based on previous studies reporting that a high dose of simvastatin formulated in a gel can stimulate cranial bone apposition and the bone formed remained for up to 22 days after dosing.⁵

These findings pose the possibility that statins can be used for local bone repair to enhance new bone formation in orthopedic indications, in surgical repair of bone defects, and in conditions such as periodontal bone disease. There are numerous advantages for the local use of statins to stimulate bone formation: first, it is an inexpensive drug to manufacture compared with recombinant proteins, such as BMPs or fibroblast growth factors; second, it has a long history of clinical systemic usage with very acceptable good toxicity profiles; and finally, it is relatively easy to incorporate into biodegradable matrices that regulate its release to enhance its effectiveness.^{17,18}

When a surgical procedure is performed for placement of dental implants, the trauma necessary for the creation of surgical defects and the presence of implants may trigger a complex process involving several cellular and extracellular events. The relationship of these events with the use of topical statins has been discussed in the literature. Titanium implants installed after topical application of simvastatin have shown abundant formation of trabecular bone, thicker in the medullary canal, and an improvement in the bone-implant interface and bone mineral density when analyzed histologically.^{3,8,20}

Lee et al¹³ showed that a 0.5-mg dose of simvastatin gel can induce a cumulative effect in new bone formation with minimal edema and without attenuation of mechanical properties, compared with the control group, suggesting that simvastatin injection is a comfortable and flexible method to initiate bone formation in areas of thin, vulnerable alveolar bone.

Mundy et al⁶ showed that lovastatin and simvastatin can specifically activate the BMP-2 promoter. Those authors used cultured bone cells from the calvaria of mice and observed that statins significantly increased the number of osteoblasts and the amount of new bone formation. A similar effect was observed in vivo when simvastatin was

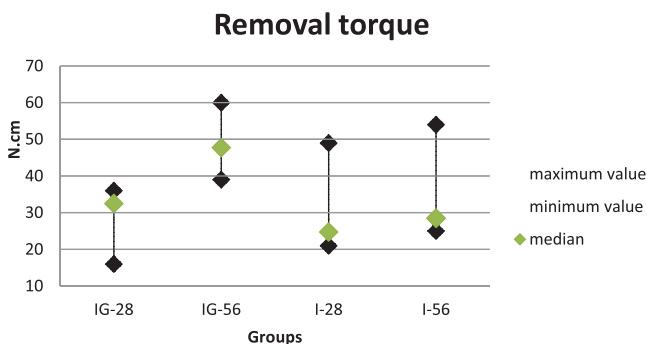


FIGURE 1. Maximum, minimum, and median values of removal torque in groups IG-28, IG-56, I-28, and I-56

injected subcutaneously over the calvaria of mice at concentrations of 1 to 10 μ M.

In our study, group IG-56 (test, 56 days) had greater removal torque values than group I-56 (control, 56 days), suggesting that simvastatin may hasten the process of implant osseointegration (Tables 1 and 2) and that the increase in the removal torque force of implants is likely to be a result of the increase in the surface area of osseointegration. However, histomorphometric studies of the bone-implant interface are required to elucidate this issue. Ayukawa et al²⁰ and Thylin et al⁵ investigated whether local simvastatin delivery affected cellular events and new bone formation in surgically created bone defects in rats. Histological and histomorphometric analyses demonstrated that local administration of simvastatin stimulates bone formation in the early stages of artificially created bone defects.^{5,20}

Our results revealed no statistically significant differences between groups IG-28 (test, 28 days) and I-28 (control, 28 days; Tables 1 and 2). However, mean removal torque force was greater, although not statistically significant, in group IG-28 than in group I-28 (Table 2). A discrepancy observed in the results for animal 1 is likely to be the reason for this statistical difference. Skoglund and Aspenberg²⁴ conducted a study in which simvastatin was either administered subcutaneously or directly applied to the fracture area in mice, with the goal of stimulating fracture repair. Simvastatin applied directly to the fracture area had a positive effect on fracture healing when biomechanical parameters were analyzed.²⁴

In the present study, topical simvastatin injected in surgical defects before implant placement promoted an increase in the removal torque force

of implants in group IG-56 (test, 56 days) when compared with groups IG-28 (test, 28 days), I-28 (control, 28 days), and I-56 (control, 56 days). Our findings indicate that removal torque force increased under the influence of simvastatin, hastening the process of osseointegration among test animals, which is in agreement with studies reporting that topical delivery of simvastatin is associated with an improvement in bone regeneration.^{4,6,14,19-22}

CONCLUSION

Our results indicate that topical administration of a 7.5-mg dose of simvastatin gel was effective in improving the torque force required to remove implants inserted in the rabbit tibia.

ABBREVIATIONS

BMP-2: bone morphogenic protein-2
HMG-CoA: 3-hydroxyl-3-methylglutaryl coenzyme A

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