Evaluation of the Effect of Simvastatin on the Progression of Alveolar Bone Loss in Experimental Periodontitis - An Animal Study

Carlos Augusto Nassar[1], Guilherme Degani Battistetti[2], Flávia Pardo Salata Nahsan[3], Joice Olegário[4], Juliana Marconato[5], Caroline Fernandes Marin[6], Daniele Marin Faccioni[6], Karine Figueiredo da Costa[7], Luciana Bill Mikito Kottwitz[8], Patricia Oehlmeyer Nassar[9]

Departments of [1]Periodontology and [2]Maxillofacial Surgery at the State University of West Parana, UNIOESTE, Cascavel, Paraná; [3]Federal University of Sergipe, Aracaju, Sergipe; Departments of [4]Endodontics and [5]Orthodontics at the State University of West of Parana, UNIOESTE, Cascavel, Paraná, Brazil; [6]Surgeon Dentist, Cascavel, Paraná, Brazil; [7]Masters Student in Dental School at State University of West of Parana (UNIOESTE) Cascavel, Paraná, Brazil; [8]Adjunct Professor of the Pharmacy School at State University of West Parana; [9]Adjunct Professor of the Discipline in Periodontology at State University of West Parana - UNIOESTE – Cascavel – Paraná - Brazil

Abstract

Aim: The aim of the present study was to evaluate the effect of simvastatin, a potent anti-inflammatory drug, on the progression of alveolar bone loss in an experimental model of periodontitis in rats.

Material and Methods: A cotton ligature was used around the lower right first molar in a submarginal position in order to induce experimental periodontitis. Sixty rats were divided into 12 groups consisting of three control groups, three simvastatin groups, three ligature groups, and three ligature plus simvastatin groups. The distance between the cemento-enamel junction and the alveolar crest was determined at the mesial root surfaces of the lower right first molars by radiographic as well as profilometric analyses.

Results: In rats of the experimental periodontitis group (ligature), alveolar bone loss was higher compared to the control groups. However, simvastatin was associated with decreased alveolar bone loss in all treatment groups with experimental periodontitis ($p < 0.01$).

Conclusion: Simvastatin treatment seems to be a beneficial and supporting therapeutic that favors protection against alveolar bone loss in a model of experimental periodontitis in rats.

Key words: Simvastatin, periodontitis, radiography

Introduction

Periodontitis is a model of leukocyte-mediated bone loss and inflammation (Van Dyke and Serhan, 2003) that has similar pathogenic features to other inflammatory diseases such as rheumatoid arthritis (RA) (Bozkurt *et al.*, 2006). There is much evidence that host inflammatory responses play an important role in periodontal tissue destruction (Nassar *et al.*, 2005). Tissue breakdown resulting from periodontal disease is partly caused by host and bacterial proteinases on periodontal tissues. Statins are potent inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), the proximal and rate-limiting enzyme of the mevalonate pathway (Endo *et al.*, 1977; Istvan and Deisenhofer, 2001). Inhibition of HMG-CoA prevents cholesterol production but also impairs the synthesis of isoprenoid lipids necessary for the prenylation of small guanosine triphosphatases (GTPases), which are critical signaling molecules that require addition of an isoprenoid tail to be active in cell membranes (Zhang and Casey, 1996; Nassar *et al.*, 2009). Statins have also been characterized by their immunomodulatory effects, as well as anti-inflammatory properties, as demonstrated by their ability to decrease plasma levels of C-reactive protein (CRP) (Kwak *et al.*, 2003;

Correspondence to: Carlos Augusto Nassar, DDS
Pernambuco Street, 593 – apartment 504
Centro (downtown) – Zip code: 85810-020
Cascavel – Parana – Brazil.
Phone number: + 55 45 32203168/ 45 91170903
Fax number: + 55 45 32203169 Email: canassar@yahoo.com

Table 1. Distribution of the groups of rats (n = 5) according to the treatment and time period of the experiment (days). Groups I and V and IX were considered control groups.

Group	Treatment	Period of treatment (days)
I	control	5
II	simvastatin	5
III	ligature	5
IV	ligature and simvastatin	5
V	control	10
VI	simvastatin	10
VII	ligature	10
VIII	ligature and simvastatin	10
IX	control	15
X	simvastatin	15
XI	ligature	15
XII	ligature and simvastatin	15

McCarey *et al.,* 2004; Chu *et al.,* 2012; Peña *et al.,* 2012). However, the mechanism by which statins reduce plasma CRP remains unknown (Arnaud *et al.,* 2005; Chu *et al.,* 2012).

The report by Pruefer *et al.* (2002) convincingly demonstrated that pretreatment with clinically relevant doses of simvastatin attenuates endotoxin-induced leukocyte rolling and transmigration in the rat mesentery. Simvastatin was administered 18 hours before endotoxin. Simvastatin therapy up-regulated endothelial nitric oxide synthase (eNOS) expression by 50% in the endothelium and decreased endothelial cell P-selectin expression. Circulating cholesterol levels were not reported in this study, but it is unlikely that acute statin therapy would alter lipid levels in rodents.

There are several studies supporting the clinical evidence of the potent anti-inflammatory and cardioprotective effects of statins (Ridker *et al.,* 1999; Kim *et al.,* 2012; Voegel and Forrester, 2013). It is possible that these acute effects of statin therapy may be related to attenuation of inflammatory processes (Lefer, 2002). Mundy *et al.*, (1999) showed that simvastatin can induce the expression of bone morphogenetic protein 2, a member of the transforming growth factor β superfamily and a key regulator of bone morphogenesis. Statins can also stimulate the expression of bone anabolic factors such as vascular endothelial growth factor (VEGF) and promote osteoblast differentiation and mineralization in MC3T3 cells (Maeda *et al.,* 2001). In addition, previous studies have demonstrated that statins inhibit the release of IL-1, IL-6 and TNF-α by up-regulating nuclear receptors and peroxisome proliferator-activated receptor (PPAR-α and PPAR-γ) (Chang *et al.,* 2005) which may represent a direct antagonist effect to cyclosporine A (CsA) (Nassar *et al.,* 2009).

The aim of the present study was to evaluate the effect of simvastatin, a potent anti-inflammatory drug, on the progression of alveolar bone loss in an experimental model of periodontitis in rats using radiographic analysis and profilometers.

Materials and methods

Animals

Sixty male Wistar rats (*Novergicus albinus*) were housed in polypropylene cages in groups of five per cage and fed standard laboratory chow (Labina, Purina, SP, Brazil) and water *ad libitum*. All protocols described below were in agreement with the Institutional Experimentation Committee of the School of Dentistry of Cascavel State University of Paraná (Cascavel, Paraná, Brazil) and National Council for Control of Animal Experimentation (Brazil).

Experimental protocols

The rats were randomly divided into 12 experimental groups with five rats each. Three groups were treated with saline solution (0.9% NaCl) and used as controls. Three groups were treated with oral daily doses of simvastatin (Novartis, São Paulo, Brazil) at 20 mg/kg of body weight. Three groups received a cotton ligature (Coats Corrente Ltda., SP, Brazil) around the lower right first molar in a submarginal position to induce experimental periodontitis, according to the methods proposed by Nassar *et al.* (2004). Anesthesia was

Table 2. Measurements (means ± standard deviations) of the distance between the cemento-enamel junction and the alveolar bone crest (pixels and mm) on the mesial surface of the mandibular first molars at 5 days.

Group (n = 5)	Treatment	Mean (pixels)	Mean (mm)
I	control	0.58 ± 0.12 A	0.325 ± 0.05 A
II	simvastatin	0.60 ± 0.12 A	0.326 ± 0.12 A
III	ligature	0.81 ± 0.23 B	0.463 ± 0.08 B
IV	ligature and simvastatin	0.78 ± 0.17 C	0.409 ± 0.04 C

Different letters represent statistically significant differences ($p < 0.01$)

Table 3. Measurements (means ± standard deviations) of the distance between the cemento-enamel junction and the alveolar bone crest (pixels and mm) on the mesial surface of the mandibular first molars at 10 days.

Group (n = 5)	Treatment	Mean (pixels)	Mean (mm)
V	control	0.66 ± 0.07 A	0.363 ± 0.07 A
VI	simvastatin	0.68 ± 0.20 A	0.365 ± 0.05 A
VII	ligature	1.41 ± 0.29 B	0.585 ± 0.11 B
VIII	ligature and simvastatin	1.19 ± 0.32 C	0.506 ± 0.03 C

Different letters represent statistically significant differences ($p < 0.01$)

obtained by intramuscular administration of 0.08 mL/100 g of body weight of ketamine (Francotar, Virbac do Brasil Ind. e Com. Ltda., São Paulo, SP, Brazil). The other three groups received the cotton ligature and were treated with simvastatin. The rats were sacrificed 5, 10 or 15 days after commencement of the experimental protocol. The distribution of the animals is summarized in *Table 1*.

Radiographic analysis

The rat mandibles were removed to determine the degree of bone loss. Standardized digital radiographic images were obtained with the use of a computerized imaging system (Sens-A-Ray 3.11, London, UK) that utilizes an electronic sensor instead of an X-ray film. Electronic sensors were exposed at 70 kV and 8 mA with the time of exposition at 0.3 impulses/second. The source-to-film distance was always set at 50 cm, and an aluminum wedge was incorporated into the electronic sensor to provide a radiographic density reference. The distance between the cemento-enamel junction and the alveolar crest was determined at the mesial root surfaces of the lower right first molars with the aid of a software package. Millimeters of bone loss on each radiograph were measured three times by the same examiner on different days in order to reduce variation in the data.

Digital images were analyzed using the Sigma-Scan 2.0 software (London, UK). Records of the distance between the cemento-enamel junction and the alveolar crest at the mesial surfaces of the mandibular first molars of the rats were taken. Data were expressed in pixels as means and standard deviations and the means of the three measurements were used for statistical analysis. Statistical evaluation was done by analysis of variance (ANOVA). Tukey's test was used to compare differences among groups. Significance level was set at 1% ($p < 0.01$).

Profilometer analysis

After taking the digital radiographic image, mandibles were thoroughly cleaned by complete removal of soft tissues, and then they were positioned and analyzed by profilometry (Starrett Sigma VB300, North Yorkshire).

Table 4. Measurements (means ± standard deviations) of the distance between the cemento-enamel junction and the alveolar bone crest (pixels and mm) on the mesial surface of the mandibular first molars at 15 days.

Group (n = 5)	Treatment	Mean (pixels)	Mean (mm)
IX	control	0.79 ± 0.20 A	0.384 ± 0.10 A
X	simvastatin	0.80 ± 0.07 A	0.383 ± 0.06 A
XI	ligature	1.39 ± 0.24 B	0.636 ± 0.07 B
XIV	ligature and simvastatin	1.31 ± 0.24 C	0.555 ± 0.14 C

Different letters represent statistically significant differences ($p < 0.01$)

Millimeters of bone loss on each radiograph were measured three times by the same examiner on different days in order to reduce errors. Data were expressed in millimeters (mm) as means and standard deviations, and the means of the three measurements were used for statistical analysis. ANOVA was used for statistical evaluation. Tukey's test was used to compare differences among groups. Significance level was set to 1% ($p < 0.01$).

Results

Clinical observations

Simvastatin had a generally favorable safety profile and was well tolerated over the 5, 10 and 15 days of the treatment. No relevant clinical manifestations were observed in rats treated with simvastatin. No alterations of either the palatal or lingual mucosa were observed in any simvastatin-treated groups.

Radiographic aspects and profilometer analysis

The satisfactory outcome of the experimental periodontitis model was confirmed, as increasing bone loss over the time periods was evident. After 5, 10 and 15 days, simvastatin-treated rats with periodontitis presented with significantly lower alveolar bone loss compared with the untreated group ($p < 0.01$), albeit increasing bone loss was significant compared with the control group in all periods ($p < 0.01$). The distance between the cemento-enamel junction and alveolar crest is reported in *Tables 2, 3* and *4*.

Considering the radiographic analysis and profilometry of mandibles of the experimental groups in all periods it may be observed that the alveolar bone loss was higher for the ligature group ($p < 0.01$) than that obtained for the control group, simvastatin group, and the simvastatin and ligature group ($p < 0.01$). However, the results obtained at all time points showed increasing alveolar bone loss for the simvastatin and ligature group compared with the simvastatin-only group ($p < 0.01$).

Discussion

The biological effects of statins on bone metabolism were first reported in 1999, when Mundy *et al.* (1999) found that these drugs were potent stimulators of *in vitro* bone formation. In fact, results of many studies strongly suggest that statins have a beneficial effect on bone health (Spolidorio *et al.,* 2007; Nassar *et al.,* 2009). When subcutaneously injected into rat calvaria, some statins stimulate bone formation as well as increase the expression of bone morphogenetic protein (BMP)-2mRNA and osteoblasts (Mundy *et al.,* 1999). Previous studies have shown that statins, which are potent cholesterol-lowering drugs, inhibit lipopolysaccharide-induced expression of pro-inflammatory genes, such as monocyte chemoattractant protein-1 (MCP)-1, iNOS, intercellular adhesion molecule-1 (ICAM-1) and interleukin-6 (Nareika *et al.,* 2007). Takebayashi *et al.* (2005) reported that low-dose atorvastatin (10 mg/d) significantly decreased the level of CRP and MCP-1 in patients with type 2 diabetes. Economides *et al.* (2004) also reported that atorvastatin improved endothelial function and decreased the levels of biomarkers of endothelial activation and inflammation in patients with type 2 diabetes mellitus (Economides *et al.,* 2004; Nareika *et al.,* 2007). Simvastatin, which is currently used to reduce cholesterol levels in humans, has protective effects against possible bone loss, as shown in *Tables 2, 3,* and *4*. No bone loss occurred before 15 days of treatment, consistent with previous results reported by Nassar *et al.,* (2009) where increased calcium levels were observed only after 15 days of simvastatin administration. One exception was the Wong and Rabie study (2005), which reported the effect of simvastatin on induction of bone formation in a trial period of less than 14 days.

Previous experimental and clinical studies have also demonstrated that statins can down-regulate both acute and chronic inflammatory processes. Early evidence for the direct vascular effects of statins was provided by clinical studies demonstrating

improvements in coronary endothelial function in patients as early as one month after the initiation of statin therapy (Lefer, 2002). This is similar to our results that demonstrate the effectiveness of the action of simvastatin in the initial induction of periodontal disease (*Tables 2* and *3*) until the conclusion of the experimental period (*Table 4*). It soon became evident that statins had potent actions on the vascular endothelium that might be mediated by eNOS. Landmark studies by Laufs *et al.* (1997; 1998) reported that statins up-regulate eNOS function under baseline conditions and after hypoxic conditions (Lefer, 2002). Laufs *et al.* (1997) reported that simvastatin and lovastatin increased endothelial cell eNOS mRNA half-life from 13 to 38 hours. Furthermore, Laufs *et al.* (1997; 1998) discovered that statins raised endothelial NOS function via inhibition of biosynthesis of L-mevalonate and the isoprenoid geranyl geranyl pyrophosphate (GGPP). The study of Kureishi *et al.,* (2000) strongly suggested that simvastatin activates the protein kinase Akt, resulting in improved endothelial function though enhanced eNOS phosphorylation and NO generation via eNOS. Kureishi *et al.* (2000) also reported for the first time that simvastatin treatment can attenuate endothelial cell apoptosis and augment angiogenesis in the ischemic rabbit hind limb model system. Since eNOS-derived NO represents a highly potent anti-inflammatory signaling pathway, the investigation of statins as anti-inflammatory agents is very logical (Lefer, 2002).

Nassar *et al.* (2009) showed that administration of simvastatin counteracted the deleterious effects of CsA on bone turnover in the absence of inflammation. These results are compatible with those reported by Ohno *et al.* (2003), who showed that treatment with cerivastatin improves CsA-induced high turnover osteopenia in transplanted bone, mainly through the inhibition of bone resorption (Nassar *et al.,* 2009). According to Lin *et al.* (2009), during systemic simvastatin use the effect of decreased bone loss had a close connection with delayed macrophage migration to the damaged bone, similar to our results *(Tables 2, 3 and 4).* Ayukawa *et al.* (2004) observed that simvastatin promoted osteogenesis around titanium implants, increasing integration and improving the natural bone formation. Kilic *et al.* (2008) showed a beneficial effect in the treatment of bone destruction in rats with systemic use of simvastatin.

Simvastatin treatment also resulted in a decrease of alkaline phosphatase in healthy animals, whereas the opposite effect was observed in the presence of periodontal inflammation. Some studies had pointed out that simvastatin enhances alkaline phosphatase activity and promotes osteoblast differentiation and mineralization (Maeda *et al.,* 2001; Seto *et al.,* 2008). However, simvastatin had no counteracting effect on the CsA-mediated decrease of alkaline phosphatase activity in healthy animals or those with periodontal disease (Nassar *et al.,* 2009). Collectively, these results suggest that simvastatin therapy has a beneficial effect on bone turnover.

Conclusion

Simvastatin treatment seems to be a beneficial and supporting therapeutic that favors protection against alveolar bone loss in a model of experimental periodontitis in rats. Additional studies are needed to understand the biological mechanisms of simvastatin in the presence or absence of inflammation.

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References

Arnaud C, Burger F, Steffens S, Veillard NR, Nguyen TH, Trono D, *et al.* Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct antiinflammatory effects of statins. *Arteriosclerosis Thrombosis and Vascular Biology* 2005; **25**:1231-1236.

Ayukawa Y, Okamura A, Koyano K. Simvastatin promotes osteogenesis around titanium implants. *Clinical Oral Implants Research* 2004; **15**:346-350.

Bozkurt FY, Yetkin Ay Z, Berker E, Tepe E, Akkus S. Anti-inflammatory cytokines in gingival crevicular fluid in patients with periodontitis and rheumatoid arthritis: a preliminary report. *Cytokine* 2006; **35**:180-185.

Chang CT, Hung CC, Yang CW, Vandewalle A, Wu MS. Cyclosporine decreases prostaglandin E2 production in mouse medullary thick ascending limb cultured cells. *Transplantation International* 2005; **18**:871-878.

Chu AY, Guilianini F, Barratt BJ, Nyberg F, Chasman DI, Ridker PM. Pharmacogenetic determinants of statin-induced reductions in C-reactive protein. *Circulation Cardiovascular Genetics* 2012; **5**:58-65.

Economides PA, Caselli A, Tiani E, Khaodhiar L, Horton ES, Veves A. The effects of atorvastatin on endothelial function in diabetic patients and subjects at risk for type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism* 2004; **89**:740–747.

Endo A, Tsujita Y, Kuroda M, Tanzawa K. Inhibition of cholesterol synthesis *in vitro* and *in vivo* by ML-236A and ML-236B, competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *European Journal of Biochemistry* 1977; **77**:31-36.

Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001; **292**:1160-1164.

Kiliç E, Özeç I, Yeler H , Korkmaz A, Ayas B, Gümüş C. Effects of simvastatin on mandibular distraction osteogenesis. *Journal of Oral and*

Maxillofacial Surgery 2008; **66**:2233-2238.

Kim YR, Park JH, Lee H-J, Bum Pyun WB, Park S-H. The effect of doubling the statin dose on pro-inflammatory cytokine in patients with triple-vessel coronary artery disease. *Korean Circulation Journal* 2012; **42**:595-599.

Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, *et al.* The HMG-CoA reductase inhibitor simvastatin activates the protein kinase akt and promotes angiogenesis in normocholesterolemic animals. *Nature Medicine* 2000; **6**:1004–1010.

Kwak BR, Mulhaupt F, Mach F. Atherosclerosis: anti-inflammatory and immunomodulatory activities of statins. *Autoimmunity Reviews* 2003; **2** : 332–338.

Laufs U, La Fata V, Liao JK. Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase. *The Journal of Biological Chemistry* 1997; **272**:31725–31729.

Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 1998; **97**:1129–1135.

Lefer DJ. Statins as potent antiinflammatory drugs. *Circulation* 2002; **106**:2041-2042.

Lin SK, Kok SH, Lee YL, Hou KL, Lin YT, Chen MH, *et al.* Simvastatin as a novel strategy to alleviate periapical lesions. *Journal of Endodontics* 2009; **35**:657-662.

Maeda T, Matsunuma A, Kawane T, Horiuchi N. Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. *Biochemical and Biophysical Research Communications* 2001; **280**:874-877.

McCarey DW, McInnes PI, Madhok R, Hampson R, Scherbakov O, Ford I, *et al.* Trial of atorvastatin in rheumatoid arthritis (TARA): double-blind, randomised placebo-controlled trial. *Lancet* 2004; **363**:2015–2021.

Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, *et al.* Stimulation of bone formation *in vitro* and in rodents by statins. *Science* 1999; **286**:1946-1949.

Nareika A, Maldonado A, He L, Game BA, Slate EH, Sanders JJ, *et al.* High glucose-boosted inflammatory responses to lipopolysaccharide are suppressed by statin. *Journal of Periodontal Research* 2007; **42**:31-38.

Nassar CA, Nassar PO, Abi Rached RS, Holzhausen M, Marcantonio E Jr, Spolidorio LC. Effect of cyclosporin A on alveolar bone homeostasis in a rat periodontitis model. *Journal of Periodontal Research* 2004; **39**:143-148.

Nassar CA, Nassar PO, Nassar PM, Spolidorio LC. Selective cyclooxygenase-2 inhibition prevents bone resorption. *Brazilian Oral Research* 2005; **19**:36-40.

Nassar PO, Nassar CA, Guimarães MR, Aquino SG, Andia DC, Muscara MN, *et al.* Simvastatin therapy in cyclosporine A-induced alveolar bone loss in rats. *Journal of Periodontal Research* 2009; **44**:479-488.

Ohno T, Shigetomi M, Ihara K, Matsunaga T, Hashimoto T, Kawano H, *et al.* Skeletal reconstruction by vascularized allogenic bone transplantation: effects of statin in rats. *Transplantation* 2003; **76**:869-871.

Peña JM, McFadyen J, Glynn RJ, Ridker PM. High-sensitivity C-reactive protein, statin therapy, and risks of atrial fibrillation: an exploratory analysis of the JUPITER Trial. *European Heart Journal* 2012; **33**:531-537.

Pruefer D, Makowski J, Schnell M, Buerke U, Dahm M, Oelert H, *et al.* Simvastatin inhibits inflammatory properties of *Staphylococcus aureus* toxin. *Circulation* 2002; **106**:2104-2110.

Ridker PM, Rifai N, Pfeffer M, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. *Circulation* 1999; **100**:230–235.

Seto H, Ohba H, Tokunaga K, Hama H, Horibe M, Nagata T. Topical administration of simvastatin recovers alveolar bone loss in rats. *Journal of Periodontal Research* 2008; **43**:261-267.

Spolidorio LC, Marcantonio E Jr, Spolidorio DM, Nassar CA, Nassar PO, Marcantonio RA, *et al.* Alendronate therapy in cyclosporine-induced alveolar bone loss in rats. *Journal of Periodontal Research* 2007; **42**:466-473.

Takebayashi K, Matsumoto S, Wakabayashi S, Inukai Y, Matsutomo R, Aso Y, *et al.* The effect of low-dose atorvastatin on circulating monocyte chemoattractant protein-1 in patients with type 2 diabetes complicated by hyperlipidemia. *Metabolism* 2005; **54**:1225–1229.

Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *Journal of Dental Research* 2003; **82**:82-90.

Voegel RA, Forrester JS. Cooling off hot hearts: a specific therapy for vulnerable plaque? *Journal of the American College of Cardiology* 2013; **61**:411-412.

Wong RWK, Rabie ABM. Early healing pattern of statin-induced osteogenesis. *The British Journal of Oral and Maxillofacial Surgery* 2005; **4**:46-50.

Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annual Review of Biochemistry* 1996; **65**:241-269.