Influence of the local application of sodium alendronate gel on osseointegration of titanium implants


Abstract. The aim of this study was to perform a comparative analysis of aspects of the osseointegration of titanium implants placed with and without the local application of a bisphosphonate agent, after 28 days in vivo. The study involved the placement of 50 commercially pure titanium implants in the middle third of the tibia of 10 rabbits, with the right tibia used as the control and the left as the test site. Sodium alendronate gel was applied locally in the test group and sterile saline solution in the control group. After euthanasia, 10 implants from each group were analyzed for maximum removal torque. The remainder of the sample was processed to obtain non-decalcified slides, approximately 30 μm thick, for histomorphological and histomorphometric analyses, including bone–implant contact (%BIC). Data were analyzed at the 5% level of significance. The removal torque values of the test group were, on average, half those obtained in the control group. The test group showed a lower %BIC and notable changes in bone quality. It is concluded that the initial events in the osseointegration of titanium implants are not favoured by the local application of sodium alendronate gel in rabbits.

Key words: bisphosphonates; osseointegration; bone remodelling; dental implants.

Accepted for publication 20 May 2015

Over the last few decades, there has been considerable growth in the demand for the replacement of lost teeth by means of therapy with dental implants and implant-supported dentures. Among other reasons, this is due to the fact that partial or complete edentulism affects a large proportion of persons of more advanced age, a population group that is growing steadily worldwide. The bone quality and quantity in these patients is frequently affected by systemic diseases, such as diabetes and osteoporosis, and they may also present lower potential bone regeneration, all of which may contribute to lower success rates in dental implant therapy. On the other hand, the desire to attain faster osseointegration is common for both health professionals and patients. This has motivated research into the development of materials and techniques to optimize the process of bone remodelling around dental implants.

During implant placement surgery, the primary stability of the implant in bone tissue is one of the aspects used to determine whether or not to apply an immediate load. In cases in which there is no primary...
stability during dental implant placement, it is recommended that the professional follows the protocol of two surgical stages, delaying functional loading for the period of osseointegration. In this scenario it becomes important to accelerate the process of osseointegration, so that the delay between implant placement surgery and re-opening for the connection of the prosthetic abutment can be reduced.

Bisphosphonates as bone biomodulators in implant dentistry was aroused because of the known ability of these drugs to inhibit the activity of osteoclasts; this inhibition activity is why the drugs are used widely in the treatment of diseases characterized by excessive bone resorption, such as osteoporosis, hyperparathyroidism, and bone metastases. Furthermore, it is known that an effect on bone formation may be expected, promoting remodeling of bone turnover. Studies have suggested that bisphosphonates may have a positive influence on bone formation and remodeling, and may consequently improve the fixation of titanium implants in humans.

Because of the severe side effects caused by the systemic use of these drugs, researchers have turned their attention to developing methods for the local delivery of these drugs to the site of interest. The intention is that the bisphosphonate will positivly influence the remodelling of bone adjacent to the implant, without causing undesirable systemic side effects. In this regard, immobilization of the bisphosphonate on the implant surface has been proposed as a way of delivering the drug locally. However, the methodology for this immobilization is complex and requires sophisticated equipment. The direct application of bisphosphonate to the surgical alveolus immediately before implant insertion would appear to be a simpler and more practical procedure; however this has not yet been tested extensively.

Therefore, the aim of this study was to propose the local application of a bisphosphonate drug (sodium alendronate) in gel form, directly to the surgical site, and to perform a comparative evaluation of aspects related to osseointegration of titanium implants inserted immediately after this application, in vivo. The hypothesis was that topically applied bisphosphonates would increase primary implant stability after 28 days.

Materials and methods

Animals and experimental groups

Ten adult male rabbits of the Oryctolagus cuniculus species, New Zealand lineage, with a mean body weight of 4.0 kg, were used in this research. The study was approved by the ethics committee of the university, and the animals received all the care stipulated by the institution.

A total of 50 implants were inserted in the sample. The right tibia was used as the control site and the left tibia as the test site. In the control group, sterile saline solution was applied to the surgical alveoli made in the right tibia of each animal. In the test group, a topical application of a 1 ml of sodium alendronate gel (10 mg/g) was administered to the surgical alveoli. The sodium alendronate gel was formulated specially for use in this study and the formulation was produced exactly as recommended by Reddy and Kumar.

Surgical procedure

After being weighed, the animals received pre-anaesthesia medication, comprising acepromazine maleate (0.2 mg/kg) and morphine sulphate (2 mg/kg), both administered intramuscularly. After approximately 10 min, the marginal ear vein of the animal was cannulated for the administration of fluid therapy with lactated Ringer solution and enrofloxacin (10 mg/kg) 20 min before surgery. Anaesthesia was induced by means of intravenous injection of ketamine chloride (10 mg/kg) and 1 mg midazolam (1 mg/kg). Epidural anaesthesia was administered with 2% lidocaine (0.25 ml/kg). After the induction of anaesthesia, the animals were shaved and antiseptic of the region was performed, including the skin adjacent to the shaved area.

Surgery began with a linear incision, measuring approximately 2 cm in extension, on the medial diaphyseal surface of the tibia. The sites where the surgical alveoli were to be cut were demarcated, with the perforations positioned 10 mm below the tibial condyle and a distance of 10 mm between perforations. Cutting for implant insertion was performed with appropriate burs, under irrigation, to a depth of 4 mm, using first a 2-mm lance-shaped bur and then a helical bur. After the cavities had been prepared, sterile gauze was introduced and kept in the surgical alveolus by compression for 1–2 min in order to absorb and stop the bleeding. This process guaranteed that the bisphosphonate gel would come into direct contact with the entire wall of the alveolus, without the interposition of blood.

For the test group, a 1-ml quantity of sodium alendronate was injected into the surgical alveolus immediately before implant placement (Fig. 1a). After this, the implants were inserted (Fig. 1b). Commercially pure titanium implants with an acid-treated surface were used (Porous Nano; Conexão Sistemas de Prótese, Arujá, São Paulo, Brazil); these were 2.2 mm in diameter and 4.0 mm long, and fabricated specifically for this study. The implants were inserted at a speed of 35 rpm, until they reached bone level. Two minutes after the surgery, the muscle and subcutaneous tissues were approximated with continuous sutures and the skin was approximated with simple, interrupted sutures, using resorbable suture thread (catgut 4–0; Johnson & Johnson/ Ethicon, USA). The region was cleaned with gauze dampened with physiological solution to remove the residues of blood clots, and the wound was covered with an occlusive dressing and a gauze bandage.

During the postoperative period, all animals received analgesic medication consisting of tramadol hydrochloride (2 mg/kg) delivered subcutaneously, every 8 h, for 3 days. Antibiotic therapy consisting of enrofloxacin (10 mg/kg) was administered via intramuscular injection every 24 h for 7 days.

Euthanasia of animals

At 28 days postoperative, the rabbits were euthanized. Each animal received pre-anaesthetic medication comprising acepromazine maleate (1 mg/kg), ketamine hydrochloride (15 mg/kg), and xylazine hydrochloride (2 mg/kg), all administered intramuscularly. After around 10 min, the palpebral, corneal, and pain reflexes were absent. With the animal in a plane of deep anaesthesia, 10% potassium chloride solution was administered intravenously until cardiac respiratory function ceased.

Removal torque measurement

The specimens were processed immediately after removal of the tibia for the measurement of the maximum removal torque of each implant. The tibias were first placed in 10% buffered neutral formalin solution; after 1 h, they were submitted to the torque removal test, thus they did not become dehydrated. The anatomical sample was carefully placed on the torque test equipment (CME; Técnica Industrial Oswaldo Filizola, Guarulhos, Brazil), which was controlled completely by the software programme DynaView Torque Standard/Pro M (Dyna Pro Dynamometers Ltd, United Kingdom), generating values automatically at a speed of 1 rpm and angular measurement of the

Please cite this article in press as: Guimarães MB, et al. Influence of the local application of sodium alendronate gel on osseointegration of titanium implants, Int J Oral Maxillofac Surg (2015), http://dx.doi.org/10.1016/j.ijom.2015.05.013
system with a resolution of 0.002°. The maximum torque measurement to begin the inverse rotation was recorded, and the mean torque values were calculated for each group.

**Histomorphological and histomorphometric analysis**

Bone blocks containing the implants were dehydrated gradually in successive concentrations of alcohol and the samples embedded in methacrylate-based resin using an EMBed-812 embedding kit (Electron Microscopy Sciences, Hatfield, PA, USA) in accordance with the manufacturer’s instructions. The blocks were then cut into slices approximately 300 μm thick, with the centre of the implant in the direction of its long axis, using a diamond-coated disc in a metallographic cutter (Model DTQ5; Pantec, São Paulo, Brazil). After this, the samples were bonded to an acrylic plate with acrylate-based cement and left to dry for 24 h before the stripping and finishing processes. The sections were reduced to a final thickness of approximately 30 μm with the use of a series of water abrasive papers (400, 600, 800, 1200, and 2400 grit; 3 M do Brasil, São Paulo, Brazil) in a polishing machine (Polipan 2; Pantec) under irrigation with water. Finally the sample was stained with fuchsin and analyzed under an optical microscope (Nikon Eclipse E200; Nikon Corporation, Tokyo, Japan).

All the bone–implant histological sections were analyzed histomorphologically in order to establish the general tissue characteristics of the osseointegration process in each group, by means of observing the neoformed bone tissue and its typical cell elements. An endeavour was made to record the regions of the implants with the strongest evidence of osseointegration and to evaluate the bone tissue covering the implant threads.

In the histomorphometric analysis, the bone–implant contact (%BIC) was determined at 50–200× magnification by means of a software programme (Image Tool for Windows, version 5.02, Department of Dental Diagnostic Science of the University of Texas Health Science Center, Texas, USA). The regions of bone–implant contact along the perimeter of the implant were subtracted from the total implant perimeter, and calculations were done to determine the %BIC.

**Statistical analysis**

The maximum removal torque values and the %BIC were compared between the groups by means of the paired Student’s t-test. The tests were considered at the 5% level of significance. IBM SPSS Statistics for Windows, version 20.0 software (IBM Corp., Armonk, NY, USA) was used for the data analysis.

**Results**

**Maximum removal torque**

Table 1 shows the maximum removal torque values in the control and test groups. The values in the test group were, on average, half those in the control group ($P < 0.001$).

**Histomorphological analysis**

Qualitative evaluation of the histological slides demonstrated that the most cervical portion of all of the implants passed through the tibial cortical bone, and the apical portion was in contact with medullary bone (Fig. 2).

In the control group, histological analysis showed bone neoformation in the areas adjacent to the implant surfaces, with regions of bone remodelling, showing evidence of a structural arrangement similar to that of the lamellar region. Close to the implant, a large quantity of voluminous osteocytes was observed located within wide gaps. Closer to the implant, immature bone trabeculae with innumerable large and voluminous osteoblasts were observed. The difference in staining – more intensely stained areas – revealed more recently formed bone tissue, which was found particularly in the regions between the implant threads (Fig. 3).

In the majority of specimens in the test group, the histological analysis showed an absence of bone neoformation in the areas adjacent to the implant surfaces, with sites of bone remodelling close to the tops of

**Table 1. Comparison of the maximum removal torque (N cm) between the study groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>N</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.86</td>
<td>5.15</td>
<td>17.9</td>
<td>10.1</td>
<td>25.3</td>
<td>10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Test</td>
<td>8.73</td>
<td>2.86</td>
<td>7.9</td>
<td>5.9</td>
<td>14.2</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation.
the spirals and in the more cervical portion of the implant. In the majority of samples, granular tissue was observed filling the spaces between the spirals (Fig. 4).

**Histomorphometric analysis**

The mean %BIC values in the control group were approximately 2.5 times higher than those in the test group, showing a statistically significant difference ($P < 0.001$) (Table 2).

**Discussion**

The influence of the use of bisphosphonates in implant dentistry has been studied extensively, both their systemic use\textsuperscript{27-29} and local use by means of direct application to the surgical alveolus\textsuperscript{3,22,23,25} or by immobilization of the medication on the implant surface.\textsuperscript{7,15,16,19-21} In this study, local application of bisphosphonate was used in order to benefit from its biologically beneficial influence on osseointegration in the absence of the possible systemic effects of this drug.\textsuperscript{8-11} This was done with the assumption that locally applied bisphosphonate would remain concentrated in the tissues around the site of application and would not be distributed systemically.\textsuperscript{31} However, in this study, the local application of a bisphosphonate had a visibly negative influence on bone remodelling around the implants inserted.

The test implants were inserted immediately after the local application of a bisphosphonate gel. Some studies have analyzed the local influence of this drug on osseointegration by means of applying it in the form of a solution.\textsuperscript{13,22,22} In the present study, the gel form was chosen in order to contain the drug in the surgical alveolus and keep it in contact with the bone walls during implant placement. The intention was to prevent bias in case the results were the same in the two groups due to the possibility of the drug not really being tested because it had completely extravasated.

The gel formulation applied in this test was prepared meticulously following the protocol described by Reddy and Kumar,\textsuperscript{24} and the properties of this gel have been tested adequately. The authors reported that sodium alendronate was distributed uniformly throughout the gel and did not demonstrate any chemical reaction with the excipients of the formula. The gel presented adequate viscosity for application by means of syringes, and did not favour the growth of microorganisms after being sterilized. This gel, tested in humans in periodontal therapy, has been shown to

---

**Fig. 2.** Position of the implants in the tibial cortical and medullary bone; magnification $20\times$.

**Fig. 3.** Bone–implant interface of the control group in the cortical bone region, demonstrating more intense bone activity close to the implant body, with the presence of recently formed bone (region of more intense colour), voluminous osteocytes in wide gaps (arrows), and large and voluminous osteoblasts in contact with the implant surface (stars); magnification $40\times$.

**Fig. 4.** Bone–implant interface of the test group in the cortical bone region, demonstrating low bone activity located almost only in the region at the top of the spirals and the most cervical region of the implant, with the presence of granular tissue filling the space between spirals (stars); magnification $40\times$.-----

Please cite this article in press as: Guimarães MB, et al. Influence of the local application of sodium alendronate gel on osseointegration of titanium implants, *Int J Oral Maxillofac Surg* (2015), http://dx.doi.org/10.1016/j.ijom.2015.05.013
have a powerful effect on the inhibition of bone resorption and to increase bone neoformation.

According to Fleisch,31 very high doses of non-nitrogenated bisphosphonates, such as etidronate and clodronate, are required in order to achieve inhibition of resorption; indeed, the same doses as those that harm physiological mineralization. The development of nitrogenated bisphosphonates, such as alendronate, resolved this problem: these are compounds up to 10 times more powerful in relation to resorption, but with the great difference in the inhibition of mineralization, and have been shown to be more adequate for purposes such as helping with osseointegration.

However, in a recent publication, Manzano-Moreno et al.32 reported that low doses of the nitrogenated bisphosphonates researched, among them alendronate, were capable of exerting their effect on osteoblasts, altering their physiology and diminishing their bone formation capacity by inhibiting their differentiation and maturation. This may explain the detrimental effects on the reparative capacity of these cells. This detrimental effect on bone remodelling would be an explanation for the worse osseointegration results with the local application of sodium alendronate found in the present study.

Möller et al.33 tested topical bisphosphonates for the prevention of bone graft resorption in implant dentistry. An inhibitory effect on bone remodelling was observed in the groups in which alendronate solution was used in comparison to the control groups. The authors suggested a toxic effect of the drug dosage as a probable reason for these results, which may also explain the results of the present study. The concentration of the drug used in this study was chosen based on the positive results obtained in the previous study of Reddy and Kumar,24 in which sodium alendronate was used in periodontal pockets. The negative results found in this study could be explained by the route of drug administration, since it was applied in bone cavities, in intimate contact with the bone marrow, which may have increased the drug toxicity to the tissues involved.

Furthermore, by means of histomorphological analysis it was demonstrated that the implants inserted were located exclusively in cortical bone tissue, because of the characteristics of the animal model used. Cuairán et al.,25 in a study on the effect of local application of bisphosphonate on miniscREW implant stability in dogs, observed significantly more cortical bone around the implants of the control group after 8 weeks than around those in which bisphosphonate was applied, contrary to the situation observed in trabecular bone. The authors concluded that the bisphosphonate had a positive effect on trabecular bone and a negative effect on cortical bone. This would corroborate the negative removal torque results for the bisphosphonate group in the present study.

When dental implants are inserted in bone tissue, damage is caused to this tissue by the use of the burs, and bone microfractures and necrosis may be generated in the entire adjacent tissue area. This damaged bone tissue must be remodelled by events of resorption and neoformation.34 According to Russell et al.,35 the injected bisphosphonate is expected to cause inhibition or restriction of osteoclastic activity. Since osteoclastic activity is closely related to osteoclastic activity, it is reasonable to assume that bone remodelling could be harmed by the presence of this drug in these conditions.35 This could explain the significantly worse histomorphometric and visibly altered histomorphological results found in the group tested in this research.

The study of Jakobsen et al.36 tested the osseointegration of endosseous implants inserted with a gap of 2.5 mm, which was filled with an alginate soaked in a bisphosphonate, at different concentrations. Both the control group and the group with a low concentration of bisphosphonate showed better fixation when compared to the other groups; the group with the highest concentration had the highest potential for inhibiting bone resorption. Thus, an increase in the fraction of bone volume can not be correlated with an increase in biomechanical fixation. This contrast clearly indicates that the beneficial effect of the drug is dose-dependent, and that the unfeasible maintenance of bone tissue may not result in the expected objective, since bone remodelling fundamentally requires the effect of resorption in order to occur.

Although a multitude of relevant studies have been reported in the literature, these studies have not used a standardized methodology for the comparison of results and have varied in the type of bisphosphonate used and in the form of presentation, administration route, animal model, secondary variables, and evaluation tests. Stadelmann et al.37 developed a model for bone remodelling around implants carrying two bisphosphonates for local release, taking into consideration the mechanical stimulus of the drug, in order to obtain predictable results. The authors related that although the present scientific results are based on empirical choices, it is possible to obtain predictability if the type of bisphosphonate, dose, and animal model adopted are taken into consideration.

There is a visible contrast between the results found in the current literature and those reported in the present study. The large majority of studies that have tested the local application of bisphosphonates have reported an improvement in implant fixation with an increase in peri-implant bone density,12,15,16,18,19,21,23 positive results regarding osteoblastic cell activity,24,39 an increase in primary stability,6,7,21 and a greater degree of bone-implant contact.13,21,40 However, it is believed that this may be due to a publication bias, favouring positive results in the group tested. It is important that all scientific reports based on correctly designed studies, without any distinction whatsoever, are published in periodicals with the highest impact factors, so that all of the information related to a tested product may be accessed by the widest possible audience. In addition, this would facilitate analysis by researchers who seek to conduct systematic reviews and meta-analyses on the subject.

In this study, both the evaluation of removal torque and the histomorphometric and histomorphological analyses showed worse results for the group in which the drug intervention was performed. This coherence between the biomechanical and histological findings reinforces the results as a whole and is the basis of the conclusions drawn. However, this was a preclinical study, in which only one type of bisphosphonate was tested at a single concentration, with analysis done promptly after 28 days in vivo. A great deal remains to be elucidated on this subject, particularly with respect to the applicability in humans, since the clinical scenario has not yet been explored in detail in the current scientific literature.
With the methodology used for this experiment and the analyses performed, it was found that the local application of sodium alendronate gel harmed the osseointegration of titanium implants installed in rabbit tibias, thus rejecting the study hypothesis. The local application of this drug acted to diminish the percentage of bone–implant contact and the maximum removal torque value, in addition to having a visibly negative influence on bone remodelling around the implants inserted.

Funding
There was no funding source for this research.

Competing interests
No conflicts of interest.

Ethical approval
The study involved animals. The institutional ethics committee considered and approved the research protocol (registered under CEUA 13/00344).

Patient consent
Not applicable.

References


Address:
Magali Beck Guimarães
Pós-Graduação – Odontologia
Pontifícia Universidade Católica do Rio Grande do Sul
Avenida Ipiranga 6681
Prédio 6
Zip Code 90619-900
Porto Alegre
RS
Brazil
Tel: +55 51 33203538
Fax: +55 51 33203626
E-mail: magaliguimaraes@gmail.com