Effect of Aluminum Oxide–Blasted Implant Surface on the Bone Healing Around Implants in Rats Submitted to Continuous Administration of Selective Cyclooxygenase-2 Inhibitors

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Purpose: The continual use of selective cyclooxygenase-2 (COX-2) inhibitors may have a negative impact on bone repair around titanium implants. Because modified implant surfaces could be considered an important strategy to increase success rates in some conditions that interfere in bone healing, the aim of this study was to investigate whether an aluminum oxide (Al₂O₃)–blasted implant surface could reduce the negative action promoted by the continuous administration of selective COX-2 inhibitors on bone healing around implants. Materials and Methods: Thirty Wistar rats received one titanium implant (machined or Al₂O₃-blasted surface) in each tibia and were randomly assigned to one of the following groups: saline (n = 14) or meloxicam (n = 16); each was administered daily for 60 days. Bone-to-implant contact (BIC), bone area (BA) within the limits of threads, and bone density (BD) in a zone lateral to the implant were examined in undecalcified sections. Results: The Al₂O₃-blasted surface resulted in significantly increased BIC in both groups, and meloxicam significantly reduced bone healing around implants (P < .05). For the machined surface, significant differences were observed for BIC (39.48 ± 10.18; 25.23 ± 9.29), BA (60.62 ± 4.09; 42.94 ± 8.12), and BD (56.31 ± 3.64; 49.30 ± 3.15) in the saline and meloxicam groups, respectively. For the Al₂O₃-blasted surface, data analysis also demonstrated significant differences for BIC (45.92 ± 11.34; 33.30 ± 7.56), BA (61.04 ± 4.39; 44.89 ± 7.11), and BD (58.77 ± 2.93; 50.04 ± 3.94) for the saline and meloxicam groups, respectively. Conclusions: The Al₂O₃-blasted surface may increase BIC; however, it does not reverse the negative effects promoted by a selective COX-2 inhibitor on bone healing around implants. Int J Oral Maxillofac Implants 2009; 24:226–233

Key words: anti-inflammatory agents, dental implants, osseointegration, selective cyclooxygenase-2 inhibitors, wound healing

Titanium dental implants have been extremely successful in the rehabilitation of total and partially edentulous patients.1,2 However, previous studies have shown that some systemic conditions can be associated with impaired bone healing around titanium implants and implant failure, such as some metabolic disease states or during pharmacologic intervention,3–5 including the use of selective cyclooxygenase-2 (COX-2) inhibitors.6

Selective COX-2 inhibitors have emerged with the objective of reducing the side effects of nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) and have been used to alleviate the chronic pain and discomfort related to rheumatoid arthritis and osteoarthritis.7 The mechanisms by which selective COX-2 inhibitors affect the bone during the healing process are not fully understood, but there is evidence to show that the COX-2 enzyme is a critical regulator of mesenchymal cell differentiation into osteoblasts and that it participates in osteogenesis.8,9 Thus, these

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negative effects of selective COX-2 inhibitors on bone metabolism may have an important impact on the prognosis of titanium implants, particularly when the drug is used on a daily basis for an extended period of time.6

The implant surface has been recognized as a critical factor for the achievement of osseointegration because of its effects on the proliferation, differentiation, protein synthesis, cellular attachment, and cell activity of osteoblasts. Some studies have reported that implant surface properties may positively influence the cell response at the cell-material interface, ultimately affecting the rate and quality of new tissue formation.10 In addition, some studies have shown that surface roughness can directly influence osteoblast adhesion, attachment, spreading, and metabolism, modifying and controlling the osseointegration process.11

According to some authors, the use of a modified implant surface could be a means to increase success rates in areas of reduced bone quality and in areas of regenerated bone.12–15 Furthermore, it has been suggested that modified implant surfaces can improve the prognosis of titanium implants in some conditions that interfere in bone healing around implants, such as diabetes and smoking.16,17 Based on these considerations, it seemed relevant to investigate whether a modified implant surface could reduce the negative action promoted by selective COX-2 inhibitors on bone healing around titanium implants in rats subjected to chronic administration of meloxicam, a selective COX-2 inhibitor.

MATERIALS AND METHODS

Animals
Thirty male Wistar rats (aged 10 weeks) were included in the study. During the experimental period, the animals were kept in plastic cages with access to food and drinking water ad libitum. Prior to the surgical procedures, all animals were allowed to acclimatize to the laboratory environment for 5 days. The protocol of the study was approved by the University of Campinas Institutional Animal Care and Use Committee (762-1).

Experimental Design
A screw-shaped titanium implant (AS Technology, São José dos Campos, SP, Brazil) was inserted into each tibia of the rats according to a method described in a previous study.18 Briefly, general anesthesia was obtained by intramuscular administration of ketamine (0.5 mL/kg). Skin was cleansed with iodine surgical soap. An incision of approximately 1.0 cm in length was made, and the bone surfaces of the tibiae were surgically exposed by blunt dissection. Under profuse saline irrigation, bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm. A screw-shaped, commercially available pure titanium implant, 4.0 mm in length and 2.2 mm in diameter, was placed until the screw threads had been completely embedded in the bone cortex (Fig 1). Two different implant surfaces were randomly used in each rat: one tibia received the implant with the machined surface and the other received the...
modified implant surface. Oxide-blasting treatments with aluminum oxide (Al₂O₃) particles (60 to 120 µm) and etching were used to modify the implant surfaces. Finally, the soft tissues were replaced and sutured. Postoperatively, the animals received antibiotic (Pentabiotic, Wyeth-Whitehall, São Paulo, SP, Brazil) given as a single intramuscular injection (1 mL/kg).

After the implant surgery, the animals were randomly assigned to two groups and were subjected to daily subcutaneous injections until sacrifice: saline (n = 14), administration of 1 mL/kg of saline solution for 60 days; or meloxicam (n = 16), administration of 3 mg/kg⁶,¹⁹–²⁴ of meloxicam (Movatec; Boehringer Ingelheim, Itapeverica da Serra, SP, Brazil) for 60 days.

**Histometric Procedure**

Sixty days after implant placement, the animals were sacrificed, the tibiae were removed, and undecalcified sections were prepared as previously described.²⁵ The blocks were dehydrated using an ascending series of ethanol (60% to 100%) and embedded in glycolmethacrylate resin (Technovit 7200; Heraeus Kulzer, Wehrheim, Germany). Subsequently, sections (20 to 30 µm) were obtained and stained using 1% toluidine blue staining. A blinded examiner, trained and calibrated in performing the measurements, separately recorded the following parameters:

- Bone-to-implant contact (BIC): percent bone tissue in direct contact with the implant surface along the threads
- Bone area within the limits of threads (BA): percent of bone tissue adjacent to implant surface, within the limits of the implant threads
- Bone density outside threads (BD): proportion of mineralized matrix in a 500-µm-wide zone lateral to the implant (Fig 2)

These parameters were measured on both sides of the implant by use of Image Pro software (Media Cybernetics, Silver Spring, MD).⁵,¹⁸,²⁶

**Statistical Analysis**

The hypothesis that there were no differences in BIC, BA, and BD between the groups was tested by two-way analysis of variance (ANOVA) (α = .05).

**RESULTS**

Data analysis demonstrated that meloxicam negatively affected bone healing around the implants. Significant differences were observed regarding BIC, BA, and BD between saline and meloxicam groups (P < .05). The results also showed that the Al₂O₃-blasted implant surface improved the BIC in both the saline and meloxicam groups (P < .05). However, the Al₂O₃-blasted implant surface did not significantly alter the negative effect of meloxicam on BIC when compared to the machined surface (P > .05). Data analysis did not show significant differences for the other parameters analyzed (BA and BD) (Tables 1 to 3). Figures 3 and 4 illustrate the histologic aspects observed in each group.
DISCUSSION

The desired clinical effects of the selective COX-2 inhibitors are a result of their regulation of prostaglandin synthesis, mainly through the COX-2 enzyme. By inhibiting the COX-2 enzyme and the subsequent production of prostaglandins, selective COX-2 inhibitors not only achieve their desired anti-inflammatory effects, but they also inhibit the increased production of agents that are necessary for bone healing to occur. Bone metabolism alterations and impaired bone repair have been observed following the administration of selective COX-2 inhibitors in many studies.²⁷–²⁹ It was recently demonstrated that the use of selective COX-2 inhibitors may also negatively influence bone healing around titanium implants after continuous administration.⁶

Table 1 Means and Standard Deviations (%) of BIC Within the Implant Threads

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant surface</th>
<th>Machined</th>
<th>Modified</th>
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<tbody>
<tr>
<td>Meloxicam</td>
<td>25.23 ± 9.29 Bb</td>
<td>33.30 ± 7.56 Ab</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>39.48 ± 10.18 Ba</td>
<td>45.92 ± 11.34 Aa</td>
<td></td>
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</table>

Means followed by different letters indicate significant differences (α = .05; ANOVA). Different uppercase letters represent significant differences between implant surfaces. Different lowercase letters represent significant differences between treatment groups.

Table 2 Means and Standard Deviations (%) of BA Within the Implant Threads

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant surface</th>
<th>Machined</th>
<th>Modified</th>
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<tbody>
<tr>
<td>Meloxicam</td>
<td>42.94 ± 8.12 Ab</td>
<td>44.89 ± 7.11 Ab</td>
<td></td>
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<tr>
<td>Saline</td>
<td>60.62 ± 4.09 Aa</td>
<td>61.04 ± 4.39 Aa</td>
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Means followed by different letters indicate significant differences (α = .05; ANOVA). Different uppercase letters represent significant differences between implant surfaces. Different lowercase letters represent significant differences between treatment groups.

Table 3 Means and Standard Deviations (%) of BD in a 500-µm-Wide Zone Lateral to the Implant Threads

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant surface</th>
<th>Machined</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>49.30 ± 3.15 Ab</td>
<td>50.04 ± 3.94 Ab</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>56.31 ± 3.64 Aa</td>
<td>58.77 ± 2.93 Aa</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by different letters indicate significant differences (α = .05; ANOVA). Different uppercase letters represent significant differences between implant surfaces. Different lowercase letters represent significant differences between treatment groups.

Fig 3 Histologic aspects observed within the limits of the threads and in a zone lateral to the implant surface in the saline group with (left) machined and (right) modified implant surfaces (toluidine blue; original magnification ×12.5).
Modified implant surfaces can positively improve the rate and quality of tissue formation around implants. Furthermore, they promote a positive impact on the osseointegration at some sites of reduced bone quality and in the presence of systemic conditions that may impair bone healing around implants. No information is available, however, regarding the effect of the implant surface on the bone healing around implants after continuous administration of selective COX-2 inhibitors. Thus, this study investigated whether the modified implant surface could reduce the negative action promoted by selective COX-2 inhibitors on bone healing around titanium implants in rats subjected to continual administration of meloxicam, a selective COX-2 inhibitor.

Data analysis confirmed the negative influence of the chronic use of meloxicam on bone healing around titanium implants ($P < .05$), and these findings are in accordance with a previous study. A number of mechanisms could explain the impaired bone healing promoted by selective COX-2 inhibitors. It has been related that COX-2, but not COX-1, has an indispensable role in bone formation during bone healing and that this enzyme regulates essential transcription factors, such as core binding factor alpha 1 (cbfa1) and osterix, a member of the Runx family of transcription factors, which are required for the osteoblastogenesis process and bone formation. Furthermore, COX-2 produces increased amounts of prostaglandin E2, which may induce bone morphogenetic proteins (BMPs) and/or cooperate with BMPs to increase the expression of cbfa1 and osterix. In addition, it has been suggested that there are synergistic interactions between cbfa1 and BMP-2 in stimulating osteoblast differentiation and that BMP-2 can induce the expression of osterix independently of cbfa1, although osterix has been proposed to be downstream of cbfa1. Alternative signaling pathways may act independently of, or in parallel with, cbfa1 during osteoblast lineage progression.

There is limited information regarding the effects of other NSAIDs on the osseointegration of implants. Reddy et al. evaluated the effect of a nonselective NSAID on bone healing around implants in four patients. After the implants were placed, the patients were assigned to receive 100 mg flurbiprofen twice a day for 3 months or no flurbiprofen (control group). Digital subtraction radiography suggested that the flurbiprofen appeared to be associated with increased bone density surrounding implants. These results are in disagreement with the findings of the present study and other studies that have suggested that anti-inflammatory agents reduce bone healing around implants. However, according to the authors of the previous study, the number of patients used in the case series was too small to allow conclusions to be drawn with regard to statistical significance and scientific relevance. Jacobsson et al. studied the effect of diclofenac on the fixation of titanium implants coated with hydroxyapatite inserted in the femora of 10 rabbits. Five animals received a daily dose of 30 mg of diclofenac for 7 days after implant placement. Three weeks after the surgery, the interface strengths were measured by pull-out test. The mean peak force for the diclofenac-treated group was 290 ± 57 N, compared
with 369 ± 37 N for the control group (P < .025). In agreement with the results of the present study, this investigation concluded that the NSAIDs negatively interfered with the bone healing around implants and that this effect was not neutralized by the hydroxyapatite coating of the implant.39 

Although these authors38,39 evaluated the effect of nonselective NSAIDs on bone healing around implants, no studies have investigated the effect of selective COX-2 inhibitors other than meloxicam,6 impeding the comparison of meloxicam’s effects with other COX-2 inhibitor agents.

With regard to the implant surfaces examined in this study, the results showed that the Al2O3-blasted implant surface improves BIC, both in the saline and meloxicam groups (P < .05). This observation is in agreement with evidence showing that Al2O3-blasted surfaces offer advantages in bone healing and play a positive role in osseous repair. Wennenberg et al40 used rabbit tibiae to evaluate BIC around Al2O3-blasted and machined surfaces at 12 weeks after implant placement. The authors observed a higher BIC for Al2O3-blasted surfaces. In addition, Wennenberg et al41 compared four surface modifications in rabbit tibiae, testing one machined and three Al2O3-blasted titanium surfaces, produced with different grit sizes. Al2O3-blasted surfaces exhibited, in general, a significantly higher BIC than machined surfaces. Stefani et al42 investigated the influence of the implant surface (machined or Al2O3-blasted surfaces) on osseointegration around implants inserted in the tibiae of rabbits who had been administered nicotine. A positive influence of the Al2O3-blasted surface on the degree of BIC was observed when compared to the machined surface, even after nicotine administration. Furthermore, other studies have also shown that modification of implant surfaces can result in significantly greater percentages of BIC and higher removal torque values in biomechanical testing and in increased BIC at earlier times.13,40,41

Since studies have demonstrated that rough surfaces presented enhanced bone anchorage in implants with machined surfaces, this could represent an advantage in conditions that impair bone healing. Some investigations have reported that modified implant surfaces could be considered an important strategy to increase success rates in areas of reduced bone quality or in areas of regenerated bone.12-15 Moreover, it has been suggested that treated implant surfaces could be important in some cases when systemic conditions may damage bone healing around implants, such as those involving smokers or diabetics.16,17

The exact mechanism by which osteoblasts produce more bone in the presence of a rough surface is not yet well understood; however, it is possible that the increased surface area may facilitate adhesion, since the adhesion of cells to biomaterial surfaces seems to represent a crucial step in several intracellular signaling pathways that direct cell viability, proliferation, and differentiation.44 In addition, the surface roughness appears to promote osteoblastic morphology in cultured MG63 osteoblastlike cells, as well as increased osteocalcin production, a marker of osteoblastic differentiation.45 Hatano et al46 also observed greater cell proliferation and alkaline phosphatase activity in cells cultivated on rough surfaces. Furthermore, it has been suggested that the rough implant surface provides optimal conditions for healing by promoting coagulum stability and maintenance of contact between the metal surface and the blood clot during the initial phase of healing.11 Therefore, the surface roughness can directly influence osteoblast adhesion, attachment, spreading, and metabolism, modifying and controlling the osseointegration process. Moreover, Harle et al47 showed that rough implant surfaces have a profound effect on the profile of genes expressed by the bone cells and suggested that improvements in the biologic activity, and possibly the clinical efficacy of these materials, could be achieved by selective regulation of gene expression mediated by controlled modification of the titanium surface.

Although the results of this study showed that the Al2O3-blasted implant surface improved BIC in both the saline and meloxicam groups (P < .05), this surface did not reverse the negative effects promoted by a selective COX-2 inhibitor on bone healing around implants. It is possible that the positive role of modified implant surfaces on osseointegration is dependent on the osteoblastogenesis process and on osteoblast differentiation and that selective COX-2 inhibitors promote a negative action in these processes by interfering with essential transcription factors that are required for osteoblastogenesis. In addition, it can be suggested that the harmful effect promoted by COX-2 was significant, thus impairing the positive role of the Al2O3-blasted implant surface and the reversal of the situation. These factors could explain why the modified implant surfaces were not able to reverse the negative effect promoted by meloxicam. However, the precise mechanisms of this pathway remain to be clarified.

Some important aspects of this study should be considered. First, COX-2–selective NSAIDs are also used in the short term to manage acute pain, inflammation, and swelling after surgical procedures. Thus, according to the findings of this study, the suggestion that COX-2–selective inhibitors should be avoided immediately postoperatively in patients...
undergoing implant placement is not possible, since the rats in this investigation received daily doses of drugs until the endpoint of the experiment (60 days). Moreover, since the implants in the present investigation were not loaded, more studies should be considered to investigate whether a greater healing time before loading may reduce the negative action of meloxicam on bone healing or increase the osseointegration promoted by the modified implant surfaces in rats taking meloxicam. Furthermore, animal models may not faithfully reproduce events in humans, and other studies should be considered to clinically evaluate the relevance of these findings.

The results presented in this study provide important scientific evidence that may influence the decision making about implant treatment. These results could have a profound clinical impact on the prognosis of titanium implants, particularly in view of the fact that selective COX-2 inhibitors have emerged with the objective of reducing the side effects promoted by nonselective NSAIDs and have been used in chronic administration to improve the chronic discomfort and pain related to some diseases, such as rheumatoid arthritis and osteoarthritis.7

CONCLUSION

Within the limits of the present investigation, the aluminum oxide–blasted implant surface promoted increased bone-to-implant contact, but it may not reverse the negative influence on bone healing around implants caused by continuous administration of a selective cyclooxygenase-2 inhibitor.

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