

Amoxicillin Administrations and Its Influence on Bone Repair Around Osseointegrated Implants

Gabriela Giro, PhD, *Joon In, PhD, †Lukasz Witek, MS, ‡Rodrigo Granato, PhD, §Charles Marin, PhD, ||Paulo G. Coelho, PhD, ¶Juliana Cama Ramacciato, PhD, #and Rogério Heládio Lopes Motta, PhD **

Purpose: The objective of this study was to evaluate the influence of 4 different amoxicillin administration protocols on osseointegration of dental implants.

Materials and Methods: Thirty-five Wistar rats received an implant in the right tibia and were divided into 5 groups (n = 7): the control group (G1), a group that received a single dose of amoxicillin suspension (40 mg/kg) hour before surgery (G2), a group that received amoxicillin suspension 1 hour before surgery and a 10-mg/kg dose every 12 hours for 3 days (G3), a group that received amoxicillin suspension 1 hour before surgery and a 10-mg/kg dose every 12 hours for 5 days (G4), and a group that received amoxicillin suspension 1 hour before surgery and a 10-mg/kg dose every 12 hours for 7 days (G5). The animals were sacrificed by anesthesia overdose 28 days after implant placement. The samples were retrieved for bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO) analyses.

Results: BIC analysis indicated 3 different statistical groups: G1 plus G2, G3, and G4 plus G5. There was no statistical difference between G1 and G2 or between G4 and G5. G3 presented lower values, with statistical difference for G1 plus G2 and G4 plus G5. Also, a statistical difference was found between G1 plus G2 and G4 plus G5. For BAFO evaluation, no statistical difference was found for the experimental groups.

Conclusion: The results of this study suggest that prolonged use of amoxicillin might have a negative effect on bone formation around implants.

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For decades, dental implants have been widely used, with high success rates, to replace missing teeth.^{1,2} However, implant failures can occur, and some factors, such as bacterial contamination during implant insertion, can cause early dental implant failure.^{3,4} The contamination

of the implant surface by bacterial biofilms during surgical procedures can lead to an inflammatory process of the hard and soft tissues, thus decreasing the implant success rate.⁵ Likewise, infections around biomaterials are very difficult to treat and nearly all

*Post Doctoral Fellow, Department of Periodontology and Oral Implantology, Dental Research Division, University of Guarulhos, Guarulhos, SP, Brazil.

†Ph.D. Graduate Student, Department of Pharmacology, Anesthesiology and Therapeutics, São Leopoldo Mandic Dental School, Campinas, SP, Brazil.

‡Ph.D. Graduate Student, School of Chemical Engineering, Oklahoma State University, Stillwater, OK.

§Professor, Postgraduate Program in Dentistry, School of Health Sciences, Unigranrio University, Duque de Caxias, RJ, Brazil.

||Professor, Postgraduate Program in Dentistry, School of Health Sciences, Unigranrio University, Duque de Caxias, RJ, Brazil.

¶Director for Research, Department of Periodontology and Implant Dentistry; Assistant Professor of Biomaterials and Biomimetics, New York University College of Dentistry, New York, New York.

#Professor, Department of Pharmacology, Anesthesiology and Therapeutics, São Leopoldo Mandic Dental School, Campinas, SP, Brazil.

**Professor, Department of Pharmacology, Anesthesiology and Therapeutics, São Leopoldo Mandic Dental School, Campinas, SP, Brazil.

Address correspondence and reprint requests to Dr Giro: Rua Carlos Gomes 2557, Araraquara, SP, Brazil; e-mail: gabi.giro@gmail.com
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infected implants can fail at short- and long-term follow-ups.⁶ The routine use of prophylactic antibiotics during dental implant surgery has been proposed for several maxillofacial surgical procedures⁷ or specific clinical situations, such as patients at risk for endocarditis⁸ or those who are severely immunocompromised.⁹ However, the use of antimicrobial agents in healthy patients and its correlation with implant failure and success rate remains poorly documented in the literature.¹⁰⁻¹²

Various prophylactic systemic antibiotic regimens have been proposed and evaluated to minimize infection after dental implant placement, including the prophylactic use and adjunctive postoperative use of amoxicillin for 7 days.^{13,14} That a single pre-emptive dose of an antibiotic might slightly decrease the failure of dental implants and the effectiveness of an antibiotic in the postoperative period has not been confirmed.¹⁵ A systematic review of antibiotic prophylaxis for dental implants has suggested that amoxicillin 2 g, given orally 1 hour preoperatively, significantly decreases failures of dental implants placed under ordinary conditions, but there are still inconsistencies in the literature related to the use of antibiotics and the decrease of early failures of dental implants and postoperative infections.¹⁶ Lack of standardization of antibiotic use for dental implants in clinical practice is related and became the main problem when systematic reviews were performed, and elucidative conclusions about the real benefits of antibiotic prescription for dental implants surgery were not achieved.¹⁷

Despite contradictory research results and protocol opinion concerning antibiotic use, rational antibiotic use has been mandatory in dental clinical practices to ensure maximum efficacy and minimal side effects for patients, such as diarrhea¹⁸ and anaphylaxis.¹⁹ Beyond the concern about success rate and avoiding infection, the use of antibiotic prophylaxis has been advocated to increase health-related quality of life during the postoperative period.^{20,21} Furthermore, evidence has shown that long-term and repetitive use of antibiotics leads to antibiotic resistance and the development of resistant strains.²² Although previous studies have evaluated the efficacy of different amoxicillin regimens in dental implant procedures,^{12-14,22} there are no studies of their effect on osseointegration and bone repair around dental implants. The objective of this study was to evaluate the influence of 4 different amoxicillin protocols and placebo administration on the osseointegration of dental implants using an animal model.

Materials and Methods

Thirty-five adult male Wistar rats (*Rattus norvegicus albinus*; weight, 400 ± 25 g) were obtained from CEMIB-UNICAMP (Centro de Bioterismo, ICLAS Monitoring/Reference Center, Campinas, Brazil), where

they were maintained under aseptic conditions, with a 12-hour light-and-dark cycle and a permanently controlled temperature of 21°C during the course of 6 weeks. Standard rat chow pellets and water were available ad libitum. The committee of ethics for animal research of São Leopoldo Mandic Dental School approved all procedures (protocol 2009/0149). Before placement of the implants, the animals were randomized into 5 experimental groups ($n = 7$ animals per group).

G1 (control group). Animals received physiologic saline 1.0 mL (0.9% NaCl) by oral administration 1 hour before surgery as a placebo.

G2. Animals received a single loading dose of 40 mg/kg of an amoxicillin suspension (Amoxil, GlaxoSmithKline, Rio de Janeiro, RJ, Brazil) by oral administration 1 hour before surgery.

G3. Animals received a loading dose of 40 mg/kg of the amoxicillin suspension by oral administration 1 hour before surgery and a maintenance dose of 10 mg/kg every 12 hours for 3 days.

G4. Animals received a loading dose of 40 mg/kg of the amoxicillin suspension by oral administration 1 hour before surgery and a maintenance dose of 10 mg/kg every 12 hours for 5 days.

G5. Animals received a loading dose of 40 mg/kg of the amoxicillin suspension by oral administration 1 hour before surgery and a maintenance dose of 10 mg/kg every 12 hours for 7 days.

SURGICAL PROCEDURE

All surgical procedures were performed under general anesthesia. The preanesthetic procedure consisted

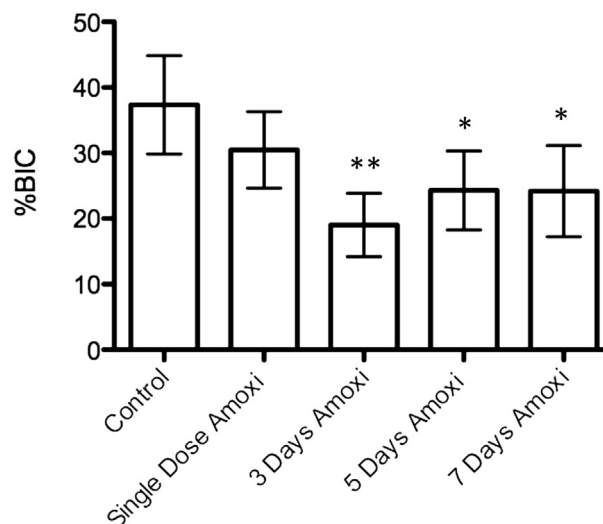


FIGURE 1. Results for BIC (mean $\pm$ 95% confidence interval). The number of asterisks indicates statistically homogeneous groups. Amoxi, amoxicillin; BIC, bone-to-implant contact.

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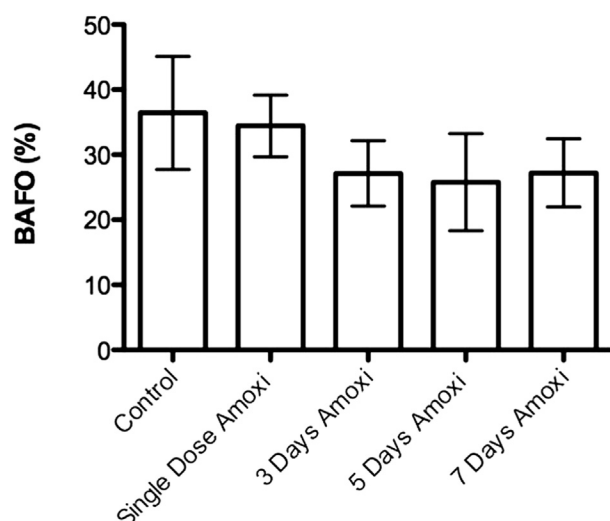


FIGURE 2. Results for BAFO (mean $\pm$ 95% confidence interval). The Amoxi, amoxicillin; BAFO, bone area fraction occupancy.

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of an intramuscular administration of xylazine chlorate (15 mg/kg; Virbaxyl, Virbac do Brasil Indústria e Comércio Ltda, Sao Paulo, SP, Brazil). General anesthesia was obtained after an intramuscular injection of ketamine chlorate (50 mg/kg; Francotar, Virbac do Brasil Indústria e Comércio Ltda). After hair shaving, skin exposure, and antiseptic cleaning with iodine solution at the surgical and surrounding areas, a 2-cm incision was performed and a full-thickness flap was reflected at the tibial diaphysis. Each animal randomly received a plateau root form implant (Bicon LLC Dental Implants, Boston, MA), measuring 4 mm in length and 2.4 mm in diameter, in the right tibia. The implant site was prepared by sequential drilling under abundant irrigation. The wound was closed with silk thread (4-0; Ethicon, Division of Johnson & Johnson Medical Limited, São Jose dos Campos, SP, Brazil). The animals were sacrificed by an anesthesia overdose 28 days after implant place-

ment. The samples were retrieved and the soft tissue was removed by sharp dissection with a periosteal elevator.

HISTOMORPHOMETRIC ANALYSIS

For histomorphometric analysis, the specimens were fixed in 10% phosphate buffered formaldehyde solution for 24 hours, washed in tap water for 24 hours, and gradually dehydrated in a series of alcohol solutions ranging from 70 to 100% ethanol. After dehydration, the samples were embedded in a methacrylate-based resin (Technovit 9100, Heraeus Kulzer GmbH, Wehrheim, Germany) according to the manufacturer's instructions. The blocks were cut by aiming the center of the implant along its long axis using a precision diamond saw (Isomet 2000, Buehler Ltd, Lake Bluff, IL) glued to the acrylic slides with an acrylate-based resin, and a 24-hour setting time was allowed before grinding and polishing. Then, the sections were reduced to a final thickness of approximately 30 μ m using a series of SiC abrasive papers (Buehler Ltd) in a grinding and polishing machine (Metaserv 3000, Buehler Ltd) under water irrigation. The sections were stained in 1% toluidine blue and examined under light microscopy. Measurements of the percentage of bone-to-implant contact (BIC) and the bone area fraction occupancy (BAFO) between threads were performed at $\times 100$ magnification (Leica DM2500M, Leica Microsystems GmbH, Wetzlar, Germany) using Image J 1.45S software (National Institutes of Health, Bethesda, MD). Statistical analysis was performed by the Kruskal-Wallis test at a 95% level of significance and the Bonferroni post hoc test using GraphPad Prism 5.0 (GraphPad, San Diego, CA).

Results

BIC analysis (Fig 1) showed that G1 presented higher values, followed by G2, G4, G5, and G3. Three different statistical groups were found, G1 plus G2,

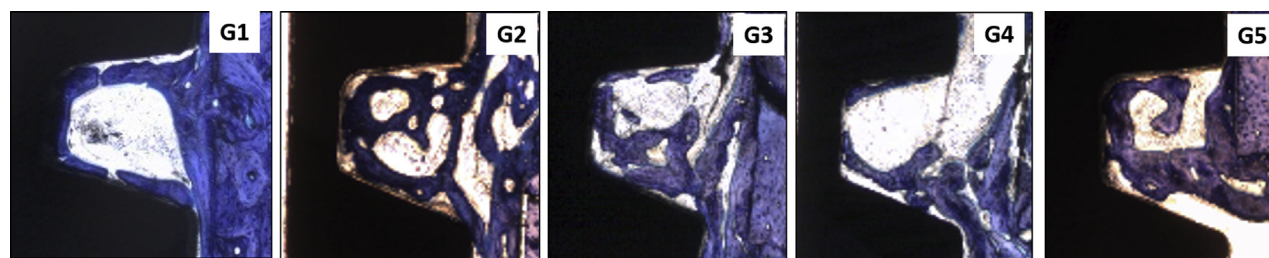


FIGURE 3. Histologic photomicrographs representing the groups in the study (toluidine blue stain; magnification, $\times 50$). G1, control group; G2, single dose of amoxicillin suspension (40 mg/kg) 1 hour before surgery; G3, amoxicillin suspension 1 hour before surgery and 10-mg/kg dose every 12 hours for 3 days; G4, amoxicillin suspension 1 hour before surgery and 10-mg/kg dose every 12 hours for 5 days; G5, amoxicillin suspension 1 hour before surgery and 10-mg/kg dose every 12 hours for 7 days.

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G3, and G4 plus G5. There was no statistical difference between G1 and G2 or between G4 and G5 ($P > .05$). G3 presented lower values for BIC, with statistical difference for G1 plus G2 and G4 plus G5 ($P < .05$). A statistical difference was found between G1 plus G2 and G4 plus G5. For BAFO analysis, G1 presented the highest value among all groups, but no statistical difference was found ($P > .05$) compared with experimental groups G2, G3, G4, and G5 (Fig 2). From a morphologic standpoint, toluidine blue staining of histologic sections showed no difference among groups. Figure 3 presents plateau photomicrographs representing each group in the study.

Discussion

Amoxicillin is a widely prescribed β -lactam antibiotic agent because of its broad spectrum, rapid and extensive absorption, and effectiveness against oral pathogens.^{3,23} Amoxicillin is a bacteriolytic agent and one of the most prescribed antibiotics in dental practice and remains the first option for the prevention of endocarditis.^{8,24} The literature extensively discusses the various reasons for implant failure and one of the most cited is that implant failure might be caused by infection associated with the surgical procedure.^{1,4,13} Therefore, the administration of amoxicillin with dental implant placement has been recommended to avoid infection that ultimately could lead to implant failure.²⁰

Several prophylactic regimens have been proposed for dental implant surgeries and no standardization or data on the influence of antibiotics on osseointegration processes can be found in the literature.^{11,15,16,20,23} Considering the results from this study, the control group (G1) presented the highest levels for BIC and BAFO, but without statistical difference from the single-dose amoxicillin group (G2), confirming a systematic review in which no differences in success rate were found and just a single prophylactic dose was recommended.³ The results presented by groups G3, G4, and G5 denote the influence of antibiotics over the osseointegration process. Bone formation can be affected temporarily by antibiotic therapy, as indicated by the early upregulation of osteocalcin, because the production of this noncollagenous protein present in bone is closely correlated with bone formation and is required to stimulate bone mineral maturation.²⁵ DNA damage has been described after the use of amoxicillin,²⁶ and an elevation of intracellular reactive oxygen species has been observed, initiating an oxidative DNA lesion. Ultimately, this lesion might be induced by the β -lactam ring present in this class of antibiotic. However, this induction achieves its plateau and begins decreasing gradually to the basal level several hours after treatment, which would explain

the decrease in BIC and BAFO for the groups that received extended treatment with amoxicillin. It is also worth noting that a single dose slightly affected osseointegration.²⁷

The use of antibiotics with no standardization is a global health problem creating resistant bacteria²⁸ and side effects, such as diarrhea,¹⁸ nausea, and vomiting.²⁹ The single dose of amoxicillin often recommended may not necessarily increase the safety of the implant placement procedure or implant success rates, and no benefits are observed clinically when postoperative doses are prescribed for regular placement of dental implants.^{3,12,30}

Considering the limitations of this study, the prolonged use of amoxicillin may have a negative effect on bone formation around implants. A single preoperative dose of amoxicillin should be used because of the minimal side effects to the host and on osseointegration. Further clinical and basic studies are warranted to elucidate these findings.

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