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# Evaluation of the effect of tacrolimus on periodontitis induced in rats

Luiz Alexandre Moura Penteado[a,b,d], Gilmara Medeiros Lucena[b], Marcílio Otávio Brandão Peixoto[b], Thiago Correia Barbosa[c], Ana Carolina de Souza Leitão Arruda[d,*], Renata Cimões[d]

[a] Federal University of Alagoas, Maceió, Algoas, Brazil
[b] University Center Cesmac, Maceió, Alagoas, Brazil
[c] North University of Paraná, Londrina, Paraná, Brazil
[d] Federal University of Pernambuco, Health Sciences Center, Recife, PE, Brazil

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ABSTRACT

*Objective:* This study was to investigate the effect of tacrolimus (FK506) on periodontitis induced in rats.
*Design:* Periodontal disease was induced in 30 Wistar rats which were then randomly divided into two groups: treatment with a daily injection of 0.9% saline (1 mL/day) and treatment with a daily injection of FK506. After periods of 10, 15 and 30 days the animals were killed and separate radiographs of the right and left hemimandibles were obtained. One calibrated examiner measured the periodontal bone support (PBS) in the images, after the following treatments: S, saline without ligature; SL, saline with ligature; T, FK506 without ligature; TL, FK506 with ligature. The data were subjected to analysis of variance (ANOVA) and the Tukey test ($p < 0.01$).
*Results:* The radiographic results were similar at all evaluation time points. The S treatment had a higher PBS averaging at 10, 15 and 30 days, which was statistically significant different compared with the SL treatment and TL treatment, but not significantly different from the T treatment. The SL and TL treatments showed no statistically significant differences between them.
*Conclusions:* Tacrolimus used for up to 30 days showed no protective or aggravating effects on alveolar bone loss.



## 1. Introduction

Periodontal disease is a pathologic process that affects the protective and/or support periodontal structures (Cortelli, Lotufo, & Sallum, 2005). The primary cause of periodontal disease is the accumulation of microorganisms organized in the form of dental biofilm (Newman, Takei, Klokkevold, & Carranza, 2006; Page & Kornman, 2000). Apical migration of the junctional epithelium and adjacent conjunctive tissue, with loss of alveolar bone structure, distinguishes periodontitis from the various forms of gingivitis (Clemons, Reynolds, Agarwal, Boughman, & Suzuki, 1990; Pihlstrom, Michalowicz, & Johnson, 2005).

During disease progression, different degrees of compromise of the periodontium may be observed, and there may be tooth loss. The scientific literature has shown that, in some situations, periodontal disease may have a significant impact on the individual's systemic condition (Lindhe, Karring, & Lang, 2003). Therefore, it may play a role as an infectious focus, contributing to cardiovascular diseases, cerebral vascular accidents, diabetes mellitus, premature birth, respiratory diseases, among others (Cury, Joly, de Araújo, Wassall, & de Araujo, 2003; Lindhe et al., 2003).

The routine therapy adopted for the treatment of periodontal disease is control of the local causative factors. This treatment is based on the mechanical processes of root scaling and planing (RSP) accompanied by effective mechanical biofilm control (brushing) by the patient. When it is not possible to reverse disease progression exclusively by means of mechanical control, there is the possibility of periodontal surgical intervention (Lascala & Moussalli, 1999).

However, there is a form of periodontal disease that affects around 2% of the population (Quirynen et al., 1995) which is characterized by rapid evolution and is frequently associated with highly virulent and resistant bacteria. In these cases, the disease is classified as aggressive periodontitis, and it has been demonstrated

* Corresponding author at: Federal University of Pernambuco, Health Sciences Center, Av. Prof. Moraes Rego, 1235, University City, Recife, PE, 50670-901, Brazil.
*E-mail addresses:* carolinalfalcao@hotmail.com, carolinalafalcao@gmail.com (A.C. de Souza Leitão Arruda).

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that this form of presentation of the disease does not respond adequately to routine conventional therapies (Armitage, 1999).

Newman et al. indicated the coadjuvant use of antibiotics for the treatment of aggressive periodontitis, and these may be systemically or locally administered, with the purpose of potentiating the effect of scaling, since there is evidence of the presence of the *Agreggatibacter actinomycetemcomitans*, strictly anaerobic microorganism (Newman et al., 2006).

However, the use of antibiotics has been shown to have limitations in some cases, in addition to the question of the possibility of bacterial resistance resulting from indiscriminate use. Thus, over the last few decades, the use of immune response modulators has emerged as an alternative treatment for cases of aggressive periodontitis. Chemically modified tetracyclines, such as doxycycline (Periostat®) and minocycline, have been shown to have this characteristic (Lotufo, Kantorski, & Zimmermann, 2003; Lindhe et al., 2003; Newman et al., 2006) cyclosporine (CsA) itself is essentially an immunosuppressor. Nassar et al. by means of an experimental study, proved that immunosuppressed rats treated with CsA presented less alveolar bone loss under conditions of induced periodontitis (Nassar, Oehlmeyer, Abi Rached, & Spolidorio, 2000). Nassar et al., also observed that there was less alveolar bone loss in animals with induced periodontal disease treated with meloxicam in a subchronic and chronic form, compared with those who were not treated; there was a reduction in the progression and intensity of periodontal disease because, according to the authors, this medication acts on the immunoinflammatory response (Nassar, Nassar, & Inagaki, 2003).

Therefore, further studies are needed to corroborate the information already available. In addition, with a view to greater power, cost reduction, ease of application, and lower toxicity must be investigated, and evidence of how other immunosuppressive drugs behave is needed.

Tacrolimus (FK506) is a macrolide antibiotic produced by *Streptomyces tsukubaensis*, available on the Brazilian market for topical or systemic use. As occurs with CsA, tacrolimus inhibits T-cell activation by blocking calcineurin activity, thereby leading to a reduction in cytokine formation. At present, it is mainly prescribed for cases of liver and kidney transplants (Silva, 2006; Yagiela, Neidle, & Dowd, 2000) .The hypothesis raised in this study is that tacrolimus may have an effect on the course of periodontal disease and may prevent and/or assist in the treatment of periodontal diseases that do not respond adequately to conventional therapy.

Using radiographs, this study evaluated the immunosuppressive effect of tacrolimus on periodontitis induced in rats, and the cytotoxicity resulting from tacrolimus therapy using biochemical and histopathological analyses.

## 2. Materials and methods

The project was submitted for evaluation to the Research Ethics Committee (REC) of the School of Biological and Health Sciences – Faculdade de Ciências Biológicas e da Saúde of FEJAL/Cesmac (registered with CONEP/CNS/SIPAR/MS on 14/12/2007 No. 25000.223512/2007-58) and was approved under Protocol No. 082A/2009.

After approval, an experimental study was performed using an animal model. The sample comprised 30 clinically healthy adult male Wistar rats from the same lineage ranging in weight from 250 to 300 g, obtained from the Vivarium of FCBS, FEJAL/CESMAC. The animal model used in this study was in accordance with the literature, in which rats are frequently used to study chronic periodontitis (Johnson, 1975; Koide et al., 1995). These animals offer some advantages, such as price and ease of handling, and they allow microbiological, macroscopic, histological and radiographic evaluation (Rêgo, de Lima Pompeu, Moreira, Naidu, & da Silva Pereira, 2010).

Throughout the experiments, the rats were maintained in 12-h (light/dark) circadian cycles, and were fed with rations (Labina – Purina®, São Lourenço da Mata/PE, Brazil) and water ad libitum (Irie, Tomofuji, & Tamaki, 2008; Sanbe et al., 2007).

To investigate the hypothesis raised as the central question of this study, periodontal disease was induced in the animals.The clinical, radiographic, and histological aspects of periodontal disease in rats are similar to those found in humans (Klausen, Evans, & Sfintescu, 1989; Koide et al., 1995; Rêgo et al., 2010). The animals were weighed to calculate the correct dose of the anaesthetic (ketamine chloride (Dopalen – Sespo, Jacareí/SP, Brazil) and xylazine chloride (Rompun – Bayer do Brasil, São Paulo/SP, Brazil)). The animals were anesthetized using doses of 0.8 mL/100 g and 0.4 mL/100 g of body weight, respectively, intramuscular injection (Nassar et al., 2003; Ricardo, Balducci, Vasconcelos, & Carvalho, 2006; Rêgo et al., 2010).

After anaesthesia the animals were placed on an operating table (Doku, Shklar, & Bugbee, 1966). The oral cavity was opened using two rings made of Chrome Nickel No. 0.07 orthodontic wire (Dental Morelli Ltda, Sorocaba – SP) fastened to the incisors (maxillary and mandibular); the tongue was then pulled with a silk suture thread No. 3-0 (Ethicon, São Paulo/SP, Brazil) in the opposite direction to the tooth that would receive the ligature (right mandibular 1st molar, 1 st RMDM).

Using a No. 5 exploratory probe (Prata Produtos Odontológicos Ltda, Caieiras – SP), temporary separation was performed between the right mandibular first and second molars; the No. 5 probe was always introduced in a standardized manner in the distal crenation of the first molar, and separation was considered complete when the top of the probe was visualized in the lingual to vestibular direction. This separation enabled subgingival insertion of the ligature (silk suture thread No. 3-0, Ethicon, triangular needle CT 1.7 cm, 45 cm long, black color, Brazil) using two curved Kelly forceps (Golgran Instrumentos Cirúrgicos e Odontológicos Ltda, São Paulo – SP), to catch the thread and place it in the interproximal space between the right mandibular first and second molars. The ligature was then stabilized with a simple knot in the mesial region of the first molar (Fig. 1).

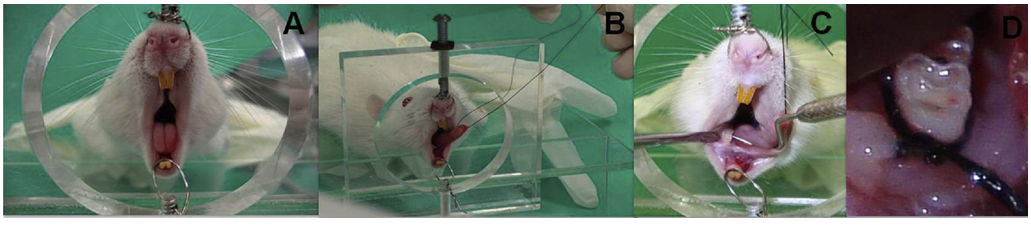


**Fig. 1.** Procedure for induction of periodontal disease: (A) positioning + opening of the oral cavity, (B) withdrawal of the tongue, (C) temporary separation of the first and second molars, and (D) subgingival placement of the ligature.