Int J Endocrinol Metab. 2012;**10**(1): 429-434. DOI: 10.5812/ijem.2555

International Journal of Endocrinology & Metabolism

Journal home page: www.EndoMetabol.com

Does the Use of Tacrolimus Influence Alveolar Bone Metabolism?

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ARTICLE INFO

Article type:
Systematic Review

Article history:
Received: 04 Oct 2011
Revised: 30 Nov 2011
Accepted: 01 Dec 2012

Keywords:
Tacrolimus
Bone
Drug

ABSTRACT

Background: Tacrolimus is commonly used in the medical area to avoid the rejection of grafted organs. Some studies have suggested that tacrolimus is an immunosuppressor that increases bone turnover and the development of severe osteopenia. In dentistry this effect may interfere with oral treatments.
Objectives: A systematic literature review to test the hypothesis that treatment with immunosuppressor tacrolimus may interfere with alveolar bone metabolism.
Search Strategy: Research in the health science databases was performed and includes articles published up to August 2011.
Selection Criteria: Studies in animals and humans using tacrolimus as an immunosuppressor and capable of interfering with alveolar bone metabolism were included.
Data Collection and Analysis: The key words used were: tacrolimus and alveolar bone or tacrolimus and alveolar bone loss or FK506 and alveolar bone or FK506 and alveolar bone loss. The articles were initially selected by title and abstract and then potentially eligible articles were read and those that fulfilled the inclusion criteria were carefully analyzed and classified (A, B and C).
Results: From a total of 745 references, only 6 articles fulfilled the eligibility criteria. Three articles were classified as A and 3 as C. In spite of the methodological differences in the 6 articles (3 animal and 3 human) tacrolimus was not found to cause damage to alveolar bone tissue.
Conclusions: In humans the results are still not conclusive. In animals: tacrolimus does not produce alveolar bone loss, whereas in humans there is no evidence that this immunosuppressor produces alveolar bone loss.

Implication for health policy/practice/research/medical education:
Monitoring of bone mineral density starting at: histopathology, densitometry, radiographs and biochemical methods, allows to determine with precision the Influence of tacrolimus on alveolar bone.

Please cite this paper as:
Dos Santos RL, Pithon MM, Lacerda MCM, Oliveira Ruellas ACD, Maia LC. Does the Use of Tacrolimus Influence Alveolar Bone Metabolism? *Int J Endocrinol Metab*. 2012;**10**(1): 429-34. DOI: 10.5812/ijem.2555

1. Introduction

There are medications capable of affecting bone metabolism and the rate of tooth movement (1). Tacrolimus (FK506) is an immunosuppressor agent isolated from Streptomyces tsukubaensis (2). It is widely used in patients who have undergone organ transplants (3). Some authors (4) have suggested that FK506 has an anti-

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DOI: 10.5812/ijem.2555

inflammatory effect, which acts primarily by interfering in T cell activation, suppressing the production of pro-inflammatory cytokines particularly TNF-α, IL-1β, IL-2 and IL6, modulating inflammation and minimizing tissue destruction and bone resorption. Studies have reported that FK506 increases bone turnover and the development of severe osteopenia (5-8), other studies have found that FK506 induces osteoclastic apoptosis (9-11).

Tacrolimus is commonly used to avoid rejection of the grafted organ, but it may cause harmful effects on bone mineral homeostasis (12) and consequently influences tooth movement (1). Therefore, the aim of this study was, by means of a systematic literature review, to test the hypothesis that treatment with tracrolimus immunosuppressor may interfere in alveolar bone metabolism.

2. Materials and Methods

For this study, bibliographic surveys were performed in the following databases: Ovid MEDLINE (1950-2011), EMBASE (1980–2011) (via DIMDI / EMBASE Alert / Elsevier / Scirus), Scopus and PubMed. All articles published up to August 2011 were included. After previous testing of some key words, four of these were used to obtain better results. The terms used in the bibliographic survey were: tacrolimus and alveolar bone or tacrolimus and alveolar bone loss or FK506 and alveolar bone or FK506 and alveolar bone loss. Other key words were not used because they did not fulfill the inclusion criteria. The references of the selected articles were assessed to identify other relevant publications. The inclusion criteria were as follows: experimental studies in animals and humans, which tested tacrolimus as an immunosuppressor capable of affecting alveolar bone metabolism; analysis of experimental data by histopathology, with/without densitometry, periapical or bite-wing radiographs and biochemical methods to measure bone loss. The exclusion criteria were as follows: studies that did not show the influence of the immunosuppressor on bone metabolism; experimental analysis with only a quantitative description of data; in vitro studies (cell cultures) ; case reports; review articles; abstracts and letters to the editor. The articles were selected by the title and abstract, without any restriction on language and those that were within the exclusion criteria were eliminated at this stage. Articles that appeared in both research databases were only considered once. After initial selection, the articles were read and those that fitted with the inclusion criteria were carefully analyzed and classified according to their level of scientific relevance (*Table 1*).

The articles were assessed and classified by two independent researchers (RLS) and (MMP). In the event of discordance between the examiners, the articles were reviewed in conjunction to obtain a decision and consensus. In cases of studies that gave rise to the need for a more succinct explanation concerning the research, the authors of the study were contacted.

Table 1. Criteria Used to Classify the Articles.

Classification (Grades)	Classification Criteria
A	Randomized controlled clinical studies in (humans) and in vivo experimental studies (non-human) with good control of variables[a], using histopathology with/without densitometry, periapical or bite-wing radiographs and biochemical methods.
B	Non-randomized controlled clinical studies in (humans) and in vivo experimental studies (non-human) with moderate control of variables[a], using periapical or bite-wing radiographs with/without densitometry, and biochemical methods.
C	Non-randomized controlled clinical studies in (humans) and in vivo experimental studies (non-human) with poor control of variables[a], using periapical or bite-wing radiographs with/without densitometry, and biochemical methods.

[a] **Control of variables**: Blinding in the data analysis, comparability among the groups, representative sample, adequate research time. Classification Criteria: (A: fulfills 3-4 variables) ; (B: fulfills 2 variables) ; and (C: fulfills 0-1 variable).

3. Results

The initial research showed 745 potentially relevant articles identified and screened for retrieval from the Ovid MEDLINE and EMBASE databases, after reading the titles, 28 articles remained. At the end, after reading the title and abstract, only 6 articles were selected in accordance with the eligibility criteria (*Figure 1*). All the articles found in the Ovid MEDLINE and EMBASE databases, selected by title and abstract, were repeated in the Scopus and PubMed databases. The articles selected were carefully

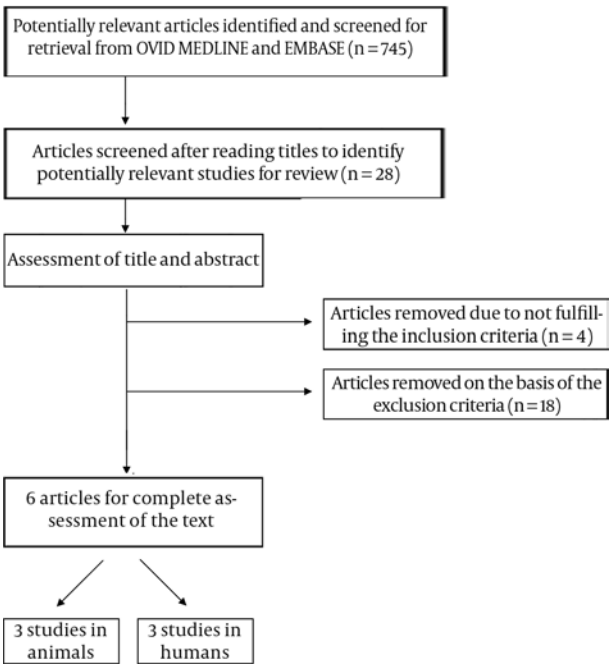

Figure 1. Flow chart of manuscripts screened through the review process.

Table 2. Study characteristics of the articles selected

Author	Sample	Medication / Dosage and Stage of Treatment	Exams Performed	Bone and Tooth Assessed	Time of Treatment	Previous Disease/ Experimental Model	Statistical Analysis	Results	Classification / Type of Study
Spolidorio, *et al.* (15)	70 male rats (50 g) : 7 groups of 10 animals distributed in groups: Control, CSA (cyclosporine) and FK506 at 2 experimental times: 60 and 120 days, and group change from CSA to FK506	Salina solution s.c.[b] injection (control) ; 10mg/Kg/day s.c. injection (CSA) ; 1mg/Kg/day s.c. injection (FK506)	Seric level of calcium; (BALP) [b]; (TRAP-5b) [b]; cytokines in serum; histology; histomorphometry	Mandibular alveolar bone and femurs; (mandibular first molars)	60 and 120 days	No	One-way (Anova) Tukey ($P < 0.05$)	BALP, TRAP-5b and increased cytokines, alveolar bone loss characterized by eroded surface of trabecular bone and diminishment of bone marrow only in group CSA[a]. Group FK506 and conversion from CSA to FK506 showed no statistical difference from the control group	A; Randomized controlled
Guimaraes, *et al.* (14)	135 male rats (100 g) : 3 groups with 45 animals	Controls: s.c. injection of 0.9% NaCl and without applying vehicle; experimental: 1 and 2 mg/Kg/day of s.c. injection of FK506	Total and differential leukocyte counts; Radiographic exam; Myeloperoxidasis activity (MPO) ; quantification of cytokines; histology	Mandibular alveolar bone; (mandibular right first molars)	5, 10, 15 and 30 days	Model of induced experimental periodontitis (cotton ligature tied around the cervical region)	One-way (Anova) Tukey ($P < 0.05$)	FK506 reduced concentration of PGE2[a] and MPO activity and prevented increase of seric levels of cytokines with both dosages. Inflammation and bone resorption was lower with FK506 compared with experimentally induced periodontitis[a]	A; Randomized controlled
Andia, *et al.* (13)	20 male rats (100 g) : 2 groups with 10 animals	Control: s.c injection of vehicle; experimental group 1 mg/Kg/day of s.c. injection of FK506	Histology; histomorphometry; structural analyses	Maxillary alveolar bone; (maxillary right first molar)	60 days	No	One-way (Anova) Tukey ($P < 0.01$)	Group submitted to application of FK506 in histomorphometry showed greater bone volume and lower number of osteoclasts compared with control group[a] with maintenance of number of osteoblasts	A; Randomized controlled
Barak, *et al.* (16)	A total of 24 women and 23 men (49.7 ± 11.4 years). Group (LC) 8 patients waiting for liver transplant; Group (PT) 22 patients submitted to transplant and submitted to CSA or FK506; 17 patients (C = control)	FK506 or CSA; (after transplant)	Panoramic X-ray (alveolar bone crest height measured from the cement-enamel junction	Maxillary and mandibular alveolar bone	Interval after liver transplant 65.7 ± 10.3 months (ranged from 2 to 156 months)	Hepatic	(ANOVA) Student's-t test. Pearson's Coefficient of Correlation Test	Alveolar bone loss was two times higher in group PT (4.67 ± 0.60) and 3 times higher in group LC (5.68 ± 0.57) compared with group C (2.47 ± 0.13) [a]. From the beginning up to present therapy with immunosuppressor, this time interval showed a tendency of inverse correlation with alveolar bone loss.	C; Clinically Controlled

Oettinger-Barak, *et al.* (17)	21 patients (52.8 ± 12 years) : 10 men and 11 women	FK506 or CSA, in 10 patients prednisone was combined, treatment with prednisone ranged from 1.5-12 years (mean of 6.47 ± 3.6 years) ; (after transplant)	Panoramic X-rays and clinical measurements (alveolar bone crest height measured from the cement-enamel junction) ; BAP [b]; IRMA [b]; DPD[b]; Pyrilinks-D ELISA; seric level of testosterone 25 (OH) D3 and PTH; densitometry (BMD) [b].	Lumbar spinal column; femur head; maxillary and mandibular alveolar bone	2.5-14.5 years (7.7 ± 4 years) ; 6 patients were in post-menopausal stage, but no women were undergoing hormonal therapy.	Hepatic; chronic moderate to severe periodontitis	Pearson's Coefficient of Correlation Test and multiple regression analysis	12 patients had osteopenia, 6 osteoporosis. 10 patients had reported low impact fractures and were defined as having severe osteoporosis. A negative correlation was found between alveolar bone loss (ABL) and treatment time with glycocorticoids, and between levels of 25 (OH) D3 and age, and positive correlation between ABL and levels of PTH. Due to the small size of sample and changes in medication therapy during the study, it was not possible to compare ABL among patients.	C; Clinically Controlled
Oettinger-Barak, *et al.* (18)	Group (LC) (46.4 ± 13.3 years) 13 patients waiting for liver transplant, 6 women and 7 men; Group (PT) (48.5 ± 13.5 years) 24 patients submitted to transplant and submitted to CSA or FK506, 13 women and 11 men and 17 patients (C = control) (48.5 ± 12.4 years), 8 men and 9 women	FK506 or CSA; (after transplant)	Panoramic X-ray (alveolar bone crest height measured from the cement-enamel junction	Maxillary and mandibular alveolar bone	104 months	Hepatic	ANOVA ($p < 0.05$) with Schaffe's modified analysis. Student's-t test and Pearson's correlation test	Alveolar bone loss was two times higher in group PT (4.57 ± 0.56) and 3 times higher in group LC (6.47 ± 0.75) compared with group C (2.73 ± 0.38) [a]. From the beginning up to present therapy with immunosuppressor, this time interval showed a tendency of inverse correlation with alveolar bone loss. The type of immunosuppressor (CSA or Fk506) did not influence alveolar bone loss (4.28 ± 0.64 mm for CSA and 4.96 ± 1.22 mm for FK506)	C; Clinically Controlled

[a] Statistically significant difference between control and experimental groups.

[b] Abbreviations: S.c, Subcutaneous; SAP, Serum bone-specific alkaline phosphatase; TRAP-5b, Osteoclast-derived tartrate-resistant acid phosphatase; BMD, Bone mineral density; IRMA, Immunoradiometric assay; DPD, Deoxypyridinoline.

read and classified according to the criteria shown in *Table 1*. This resulted in six reviewed articles, three articles were classified as having a level of scientific relevance as A and three articles as C. In the sample there were three studies conducted in animals (13-15) and three in humans (16-18), with points and important methodological features shown in *Table 2*.

The articles selected showed that tacrolimus does not cause significant deleterious effects on the alveolar bone tissue in animal studies, but in the studies in humans there was no evidence that this immunosuppressor could cause alveolar bone loss.

4. Discussion

There is increasing clinical use of tacrolimus, at present considered the basic immunosuppressor drug in over 80% of transplants (19), in addition to being used in treatments for atopic dermatitis and vitiligo, among other dermatological disorders (20).

When considering that the majority of patients who used this immunosuppressor drug require dental treatment, knowledge about its action on bone tissue will allow doubts to be cleared up with regard to performing dental procedures, such as periodontal and orthodontic procedures in specific cases, since the alveolar bone is directly involved. The articles selected (13-18), showed that tacrolimus does not stimulate alveolar bone loss. Three non-randomized controlled clinical articles were classified as C. The first non-randomized controlled clinical study (18) classified as C was conducted in patients who had undergone liver transplants with the presence of moderate chronic to severe periodontitis. Deleterious effects on bone tissue were observed during the follow-up period. However, according to the authors (18), some women patients were in the post-menopausal stage and they were not receiving hormonal therapy. Furthermore, the reduced size of the sample and the changes in medication therapy during the course of the study increased the variables of this study. The association of other medications with tacrolimus made it impossible to assess it individually. The authors (18) reported having associated prednisone, a potent corticoid, with tacrolimus or cyclosporine in 10 patients, but it is not clearly indicated in which patients. The glycocorticoids are the most common cause of secondary osteoporosis, particularly affecting the trabecular bone (21), which could influence the results found. As a control group, the authors used the same group that received the drug and at the end of the experiment, they compared the initial and final assessments. Therefore, this did not allow a comparison with healthy patients. In the first (18), as well as in the second (16) and third study (17), the assessment method used was not satisfactory. In these studies (16-18) the authors used a panoramic radiography to measure the alveolar bone, but the radiographs of choice were periapical and bite-wing radiographs, as they show fewer distortions. The studies discussed in the second and third articles (16, 17) were also conducted using patients who had undergone liver transplants. They showed that there was diminishment of alveolar bone loss after the transplant associated with the beginning of the treatment with tacrolimus or cyclosporine. On the other hand, it is not clearly indicated which patients only received tacrolimus. This affected the isolated analysis of the effects of tacrolimus on bone tissue. According to the authors of these studies (16, 17), not only the immunosuppressor effect, but also recovery of the hepatic function seems to have the potential to invert the condition of bone loss.

Three articles were classified as A, and they were all randomized controlled studies in rats (13-15). The first (15) compared the effect of tacrolimus and cyclosporine on the bone and showed that differently from cyclosporine, tacrolimus did not cause alveolar bone loss. The cyclosporine dosage used (10 mg/Kg/day) was higher in comparison with tacrolimus (1 mg/Kg/day), but if one considers that tacrolimus is 10 to 100 times more potent than cyclosporine (22), the relationship between dosage x potency becomes equivalent and therefore comparable.

The three articles used quantitative histology (13-15) as one of the exams to verify the effect of tacrolimus on bone tissue. This exam is fundamental for the classification of these studies as A. The quantitative histological exam, in turn, provides the best parameter to show the effects of the drug on the tissue when compared with the subjectivity of qualitative exams.

The second study (14) used a model of induced experimental periodontitis. This study showed evidence of a reduction in alveolar bone loss and diminishment of the inflammatory process (4) in the face of induced periodontitis, through a reduction in the concentration of PGE2 and myeloperoxidase activity. In addition to preventing the increase of seric levels of IL-1β (50.4 ± 21 pg/mL), IL-6 (26.5 ± 14 pg/mL) and TNF-α (51.5 ± 3 pg/mL) in the two dosages tested, 1 and 2 mg/Kg/day. According to the authors (14), there appears to be a potential anti-inflammatory action of FK506 on periodontitis, however further studies could explain this hypothesis better, which would be extremely important in the periodontal treatment of transplant patients. In histology and histomorphometry, the third article (13) showed a preventive capacity of tacrolimus against alveolar bone loss, showing a reduction in the number of osteoclasts and maintenance of the number of osteoblasts. This led to a greater volume of bone when compared with the control group, however the authors themselves emphasized the need for further studies that could reiterate the bone-inducing capacity of tacrolimus on alveolar bone tissue. The studies conducted in animals (13-15) showed a better methodology design, nevertheless, there is a lack of studies with well-designed methodologies, particularly in humans to assess this immunosuppressor agent due to the association between different medications, comparability and variability of groups. Although studies in animals have shown favorable results with regard to not

stimulating alveolar bone loss, it is necessary to conduct future well-designed clinical studies to support the safe use of this drug as an immunosuppressor agent that does not cause negative effects on alveolar bone homeostasis. Within the limitations of this study, it can be concluded that: in animals the tacrolimus does not cause alveolar bone loss. In the studies in humans: there is no evidence that this immunosuppressor agent can cause alveolar bone loss. However, there is a lack of methodologically well-designed studies to support its real action in human bone tissues.

Acknowledgements

We would like to thank the Department of Orthodontics and Pediatric Dentistry of Federal University of Rio de Janeiro (UFRJ) and CAPES for the research funding.

Financial Disclosure

None declared.

Funding/Support

None declared.

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