UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE ODONTOLOGIA DE ARARAQUARA

CARLOS AUGUSTO NASSAR

Avaliação morfométrica e estereométrica dos tecidos periodontais de ratos imunossuprimidos por Tacrolimus (FK506)

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Tese apresentada ao Programa de Pós-Graduação da Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista para a obtenção do título de Doutor em Periodontia.

Orientador: Prof. Dr. Luis Carlos Spolidorio

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"pela certeza de que para todos os acontecimentos da vida, bons ou ruins, existe uma razão norteada pela justiça divina."

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SUMÁRIO

INTRODUÇÃO

Os transplantes de órgãos, hoje corriqueiros, representam o coroamento de séculos de aperfeiçoamento da cirurgia, concomitante ao aprofundamento dos conhecimentos dos mecanismos de rejeição dos transplantes, que estão associados ao crescente desenvolvimento das técnicas e conceitos da imunologia e, do entendimento sobre os genes seus produtos e funções. Simultaneamente ao enriquecimento dos conhecimentos dos fenômenos biológicos fundamentados na biologia molecular, o desenvolvimento de drogas imunossupressoras tem propiciado sucesso dos transplantes e aumento na sobrevida dos transplantados. A necessidade de se evitar a rejeição levou ao desenvolvimento de novas drogas imuno-moduladoras e as pesquisas sobre as maneiras de se introduzir tolerância aos tecidos transplantados, métodos que na verdade têm uma aplicação mais geral no tratamento de várias doenças imunes, como o dano tecidual imuno-mediado na hipersensibilidade e na auto-imunidade (SPOLIDORIO et al., 2004).

Na última década ocorreram avanços substanciais na compreensão de eventos que controlam o sistema imune e dos efeitos seletivos das drogas, capazes de alterar a função imune produzindo não apenas imunodeficiências, mas também uma imunopotencialização. Os agentes mais comumente utilizados na imunoterapia sistêmica são os esteróides, ciclofosfamida, azatioprina, mofetil micofenolato, metotrexato, sirolimus (rapamicina), anticorpos monoclonais, ciclosporina (CsA) e tacrolimus (FK506) (DUNN et al., 2001; van MOURIK e KELLY, 2001; DEBRAY et al., 2003; MENGARELLI et al., 2003).

Há alguns anos junto com o aprimoramento de técnicas cirúrgicas, as drogas imunossupressoras, principalmente às de última geração, propiciaram de modo incontestável avanços significativos no sucesso na inibição da rejeição de transplantes. O grande sucesso dos transplantes culminou com a introdução da terapêutica imunossupressora com CsA, por Borel et al., em 1976, sendo atualmente substituída pelo FK506, que apresenta uma potência maior e com menores efeitos indesejáveis (OTT et al., 2003).

A CsA é um polipeptídeo cíclico hidrofóbico e lipofílico, composto por 11 aminoácidos de fórmula C_{62} H_{111} O_{12} (peso molecular 1202.6 kDa) e isolado de

duas espécies de fungos *Trichoderma polysporum rifai e Cylindrocarpo lucidum* (DALEY e WYSOCKI, 1984). Quando descoberta, foi inicialmente utilizada como agente antifúngico, sendo produzida comercialmente a partir da cultura do fungo *Tolyplocadium inflatum Gams* (BOREL et al., 1976; SEYMOUR e JACOBS, 1992). Após aprovação pelo Food and Drugs Administration , em 1985, tem sido empregada como um dos principais agentes imunossupressores em indivíduos órgãos–transplantados (DUNN et al., 2001). Em 1984, um novo agente imunossupressor, FK506, foi descoberto no Japão e usado pela primeira vez em 1989. O FK506 é um macrolídeo produzido pela fermentação com *Streptomyces tsukubaensis,* e agiria nas células mediadoras de imunidade por inibição nas sínteses de citocinas nas concentrações de linfócitos CD4 (BADER et al., 1998).

O FK506 possui ação de inibição de síntese de citocinas nos linfócitos CD4, sendo que age também na resposta imune através da inibição da formação de linfócitos-T citotóxicos responsáveis pela rejeição de enxertos, da supressão da ativação de células-T por inibir a produção de linfocinas como interleucina-2(IL-2), receptor de IL-2 e interferon gama, inibindo ainda, a ativação de células-B e bloqueando a transcrição de fator de necrose tumoral alfa (BADER et al., 1998; OETTINGER-BARAK et al., 2001). O FK506, entretanto, não inibe a proliferação secundária de células ativadas em resposta a IL-2, nem a droga interfere com a apresentação do antígeno ou modifica a função do fagócito mononuclear ou da célula Natural killer (PETERS et al., 1993; SPENCER et al., 1997; PLOSKER e FOSTER, 2000). Alguns dos efeitos do FK506 podem também ser mediados através da modulação de atividades celulares tais como a inibição da ativação de óxido nítrico sintase (KAIBORI et al., 1999; HAMALAINEN et al., 2002) (uma enzima envolvida na geração dos radicais livre de oxigênio que causam subseqüentemente danos aos tecidos) e indução de apoptoses (MIGITA et al.,1995, 1997, 1999).

O seu uso foi estabelecido como uma alternativa de droga imunossupressora em substituição a CsA, sendo que possui uma atividade imunossupressora até 100 vezes maior que a própria CsA (ZICCARDI et al.,, 1991). Entretanto, o FK506 produz efeitos secundários indesejáveis incluindo

nefrotoxicidade, neurotoxicidade e indução de estados de diabetes (JAMES et al., 2000), principalmente quando associado ao uso de esteróides (FINNI et al., 2004).

O FK506 é rapidamente absorvido no trato gastrintestinal (PLOSKER e FOSTER, 2000; SCOTT et al., 2003), sendo a taxa e a extensão da absorção reduzida na presença do alimento (PETERS et al., 1993; SPENCER et al., 1997; PLOSKER e FOSTER, 2000), demonstrado nos estudos em animais, que indicaram que o FK506 está distribuído extensamente na maioria dos tecidos incluindo os pulmões, o baço, o coração, o rim, o pâncreas, o cérebro, o músculo e o fígado (PETERS et al., 1993; VENKATARAMANAN et al., 1995; SPENCER et al., 1997; PLOSKER e FOSTER, 2000; WALLEMACQ e VERBEECK, 2001; CHRISTIANS et al., 2002).

O FK506 submete-se ao metabolismo extensivo no fígado, com menos de 1% de droga inalterada excretada na urina e metabolizada também na mucosa intestinal (VENKATARAMANAN et al., 1995; PLOSKER e FOSTER, 2000). Os dados animais indicam que a excreção biliar é a rota principal para a eliminação dos metabólitos, com eliminação fecal em 90% da dose administrada (PLOSKER e FOSTER, 2000; SCOTT et al., 2003).

Aventa-se a hipótese que fatores como dosagem, biofilme dentário, idade e gênero do paciente, assim como, fatores genéticos, tratamento concomitante com outras drogas, possam ser fatores de risco, isto é, estar associados com aumento da prevalência, da extensão ou da severidade do aumento gengival induzido pela CsA (SEYMOUR et al., 2000).

Recentemente Montebugnoli et al. (2000) e Spolidorio et al. (2004), verificaram em humanos e modelos experimentais, respectivamente, que o tempo de tratamento com CsA poderia influenciar positivamente na involução do aumento gengival. Essa informação abre outro cenário de investigação pouco explorado em humanos e em animais de laboratório. Entretanto, há relatos na literatura que os ossos de maneira geral assim como o processo alveolar (EPSTEIN, 1996; HOFBAUER et al., 1999; HOFBAUER e HEUFELDER, 2000;

FU et al., 2001; SHEN et al., 2001) e o cemento (AYANOGLOU e LESTY, 1997; AYANOGLOU, 1998, 1999) são acometidos pela CsA desequilibrando as suas homeostases.

Ainda são escassos os relatos da ação do FK506 sobre os tecidos gengivais e no processo alveolar, sendo que em diversos tratamentos, principalmente em transplantes de fígado, ocorre a substituição da CsA por essa droga imunossupressora, para que se possam reduzir os efeitos indesejáveis (JAMES et al., 2000).

Atualmente existem poucos relatos de crescimento gengival induzidos pelo uso do FK506 (ADAMS e FAMILI, 1991; OETTINGER-BARAK et al., 2001), mas na realidade, essas alterações são atribuídas a outras drogas, como a prednisolona e nifedipina, que são administrados concomitantemente com o FK506. Sugere-se que a substituição da CsA pelo FK506 possa reduzir significativamente esse efeito indesejável (KOHNLE et al., 1998; JAMES et al., 2000, 2001; HERNANDEZ et al., 2000; THORP et al., 2000).

Os dados apresentados na literatura da ação do FK506 sobre o tecido ósseo, são incertos e conflitantes. Alguns trabalhos experimentais mostram perda óssea (CVETKOVIC et al., 1994; ROMERO et al., 1995; PARK et al., 1996; CAYCO et al., 2000; STEMPFLE et al., 2002), enquanto que em outros trabalhos, o FK506 exerceu efeitos favoráveis no metabolismo ósseo, promovendo diferenciação osteoblástica, mantendo, assim, a densidade mineral óssea normal em modelos experimentais (INOUE et al., 2000; GOFFIN et al., 2002).

PROPOSIÇÃO

Baseado nas informações, anteriormente citadas, pode-se aventar alguns questionamentos: a administração do FK506 em ratos induz aumento gengival? Como se comporta a lesão gengival com o decorrer do tempo de tratamento? Partindo da premissa que o FK506 induz perda óssea, pode-se questionar se esse processo evolui proporcionalmente com o tempo de tratamento. E a eventual alteração do metabolismo ósseo está na dependência do aumento do número de osteoclastos ou nas disfunções de Ca^{2+} e fosfatase alcalina?

A partir dos dados da literatura e desses questionamentos, acima citados, o presente trabalho terá como objetivos:

- Determinar através de morfometria as características do epitélio e tecido conjuntivo da gengiva de ratos tratados com FK506 por vários períodos.
- Determinar a concentração sérica de Ca^{2+} e fosfatase alcalina em todos os períodos de tratamento, dos ratos tratados com FK506.
- Determinar através da estereometria a densidade volumétrica do osso de ratos tratados com FK506.
- Determinar as dimensões morfométricas do cemento da região de primeiros molares tratados por vários períodos com FK506.
- Determinar a concentração sérica de glicemia em todos os períodos de tratamento, dos ratos tratados com FK506.

TACROLIMUS: A OVERVIEW WITH RELATION AT PERIODONTAL DISEASE

Submetido à publicação no periódico Oral Diseases

Tacrolimus: An overview with relation at periodontal tissue

Carlos Augusto Nassar, Patricia Oehlmeyer Nassar, Denise Carleto Andia, Morgana Rodrigues Guimarães, Luis Carlos Spolidorio.

Abstract

Introduction: In the last years, the improvement of surgical techniques and the use of the immunosuppressive drugs, mainly those of the last generation, have propitiated significant advance and success in preventing rejection of transplants. The molecular mechanisms of the inhibition of T cell activation by tacrolimus are well understood. However, a frequent complication was reported in patients following organs transplant that were treated with tacrolimus. It was observed the development of side effects.

Objective: This paper reports an overview of the action of tacrolimus considering the relation and effects in the periodontal tissue.

Discussion: Some works have evaluated the skeletal effects of tacrolimus in humans or in animals, and the results obtained are uncertain and conflicting. Both cardiac and liver transplant recipients and animals have sustained rapid bone loss with tacrolimus-based immunosuppression. Other works have noted that bone loss decreases with tacrolimus and this fact has a favorable effect in bone metabolism, because it induces the differentiation of the osteoblastic, maintaining the normal bone mineral density in experimental models.

Conclusion: The results indicate that the use of tacrolimus may be beneficial for suitable patients with marked gingival overgrowth, but the action of the drug on bone tissues are uncertain and conflicting.

Keywords: tacrolimus; gingival tissue; alveolar bone; side effects

Introduction

In the practical clinic, the organ transplantation aims to improve a functional deficit, unless the donor and the recipient be genetically identical, the antigens of graft may provoke a reply of immunologic rejection. A transplant can stimulate all the mechanisms (specific and non-specific) of the humoral and cellular immunity (Dunn *et al.*, 2001).

During the last decade, substantial advances were obtained to understand the events that control the immune system and the selective effects of drugs that may alter the immune function, producing immunosuppression. The agents more commonly used in the systemic immunotherapy are steroidal drugs like cyclofosfamid, azathioprine, mycophenolate mofetil, metotrexate, sirolimus (rapamycin), monoclines antibodies, cyclosporine-A (CsA) and tacrolimus (FK506) (Dunn *et al.*, 2001; van Mourik and Kelly, 2001; Debray *et al.*, 2003; Mengarelli *et al.*, 2003).

In the last years, the improvement of surgical techniques and the use of immunosuppressive drugs, principally of the last generation, provided significant advance and success in the inhibition of the rejections of the transplants (Mengarelli *et al.*, 2003).

The molecular mechanisms of the inhibition of T cell activation by tacrolimus have been understood (Shibasaki *et al.*, 2002). The cytokine synthesis may be inhibited by Tacrolimus and this drug influences the formation of cytotoxic T-lymphocytes responsible for graft rejection. The engagement of T cell receptor with MHC/peptide normally provokes a calcium-dependent intracellular signal that results in an activation of the calcium/calmodulin-dependent phosphatase calcineurin. This leads to the dephosphorylation of NF-AT, allowing the translocation into the nucleus, where it enhances the binding of a transcription factor of the gene encoding for pro-inflammatory cytokines such as IL-2, IL-3, IL-4, IFN-γ and TNF-α. Tacrolimus enters in the cytoplasm and forms complexes with their immunophilin, called tacrolimus binding protein (FKBP-12). The tacrolimus-immunophilin complexes inhibit calcineurin activity and hence prevent nuclear translocation of NF-AT and cytokines gene transcription (Bader

et al., 1998; Oettinger-Barak *et al.*, 2001). The use of tacrolimus was established as an alternative in substitution to CsA, due to the biggest immunosuppressive activity, 500 times more efficient than CsA (Ziccardi *et al.*, 1991). However, a frequent complication was reported in patients after organs transplant and treated with tacrolimus. These patients showed the development of nephrotoxicity, neurotoxicity and induction of diabetes state (James *et al.*, 2000).

According our knowledge, there are few studies about the effect of tacrolimus on gingival tissues and cementum (Adams and Famili, 1991; James *et al.*, 2000; Oettinger-Barak *et al.*, 2001).

Some works have evaluated the skeletal effects of tacrolimus in humans or in animals. Data presented in the literature considering the action of tacrolimus on bone tissues are uncertain and conflicting. Both cardiac (Stempfle *et al.*, 1998) and liver (Park *et al.*, 1996; Cayco *et al.*, 2000; Stempfle *et al.*, 2002) transplant recipients or animals (Cvetkovic *et al.*, 1994; Romero *et al.*, 1995) have sustained rapid bone loss with tacrolimus-based immunosuppresion. However, other works suggesting a decreasing in the bone loss with tacrolimus treatment and this fact has a favorable effect in the bone metabolism, promoting differentiation of the osteoblastic, maintaining the normal bone mineral density in experimental models (Inoue *et al.*, 2000; Goffin *et al.*, 2002).

Pharmacodynamic Properties

Tacrolimus (FK506), a macrolide immunosuppressant, inhibits cellular and humoral immune responses via several mechanisms of action. The principal effect involves the inhibition of calcineurin, a serine-threonine phosphatase (Fig. 1). Inactivation of calcineurin via complex formation with the immunophilin FK506 binding protein 12 (FKBP12) prevents the translocation of the transcription factor of activated T cells that promotes interleukin-2 (IL-2) mediated proliferation of helper T cells (Plosker *et al.*, 2000; Scott *et al.*, 2003). Tacrolimus may suppress T-cell activation inhibiting the production of lymphokines, such as IL-3, IL-4, IL-6, alpha tumor factor necrosis (TNFα), beta tumor factor necrosis (TNFβ) and

gamma interferon (INFγ). Tacrolimus inhibits B-cell activation through its action of T-cells and by blocking the transcription of the alpha-TNF gene (Bader *et al.*, 1998; James *et al.*, 2000; Scott *et al.*, 2003) (Fig. 2). However, tacrolimus does not inhibit the secondary proliferation of activated cells in response to IL-2, and does not interfere with antigen presentation or modify mononuclear phagocyte or natural killer cell function (Peters *et al.*, 1993; Spencer *et al.*, 1997; Plosker, *et al.*, 2000). Some effects of tacrolimus may also be mediated by modulation of cellular activities, such as inhibition of nitric oxide synthetase activation (Kaibori *et al.*, 1999; Hämäläinem *et al.*, 2002) (an enzyme involved in the production of oxygen-free radicals that cause tissue damage) and apoptosis induction (Migita *et al.*, 1995; Migita *et al.*, 1997; Migita *et al.*, 1999).

Absorption and Distribution

Tacrolimus may be absorbed rapidly, but incompletely, in the gastrointestinal tract. The peak of tacrolimus concentration in whole-blood (Cmax) by oral administration is obtained after 1-2 hours, approximately. (Plosker *et al.*, 2000; Scott *et al.*, 2003).

The oral bioavailability of tacrolimus is poor, with average bioavailability of 25% (range 4-93%), and is similar between adult (25%) and pediatric (31%) transplant recipients (Venkataramanan *et al.*, 1995; Plosker *et al.*, 2000; Wallemacq *et al.*, 2001; Christians *et al.*, 2002; Scott *et al.*, 2003). The rate and the extent of tacrolimus absorption are reduced in the presence of food (Peters *et al.*, 1993; Spencer *et al.*, 1997; Plosker *et al.*, 2000). A study with 15 healthy volunteers was realized with a single oral dose of tacrolimus, 5mg, and showed that food had a clinically significant effect in reducing the relative bioavailability, as well as slowing the absorption, but did not influence the half-life. (Bekersky *et al.*, 2001a; Staatz & Tett, 2004).

Other studies investigated the effect of meal timing. Bekersky *et al.* (2001b) analyzed four treatments: I- fasting for 10 hours; II- ingestion 1 hour before breakfast; III- ingestion after breakfast; IV- ingestion 1.5 hours after

breakfast. Tacrolimus absorption in the fasting state (I) had a greater relative bioavailability than other treatments. The AUC averaged 312, 276, 205 and 203 μg/ h/L were employed for treatments I, II, III and IV, respectively. Absorption was significantly prolonged after meal.

Animal studies indicated that tacrolimus is widely distributed in most tissues, including lungs, spleen, heart, kidney, pancreas, brain, muscle and liver (Peters *et al.*, 1993; Venkataramanan *et al.*, 1995; Spencer *et al.*, 1997; Plosker *et al.*, 2000; Wallemacq *et al.*, 2001; Christians *et al.*, 2002). Tacrolimus may cross the placenta with umbilical cord plasma concentrations that are approximately one-third of those in maternal plasma. In addition, levels in breast milk were reported to be similar to those observed in the plasma (Venkataramanan *et al.*, 1995; Wallemacq *et al.*, 2001).

Metabolism and elimination

Tacrolimus is metabolized, extensively, by CYP3A isoenzymes (mainly) in the liver and intestinal wall, with < 0.5% of the parent drug appearing unchanged in the urine or excrements. Expression of these enzymes varies widely. The CYP3A subfamily consists of four isoforms: CYP3A4, CYP3A5, CYP3A7 and CYP3A43. These isoforms overlap substrate specificity, then, it is difficult to separate the relative contributions to the metabolism of the drug (Staatz & Tett, 2004). Tacrolimus undergoes extensive metabolism in the liver, with less than 1% of unchanged drug excreted in the urine, and it is also metabolized in the intestinal mucosa, in a minor extension (Venkataramanan *et al.*, 1995; Plosker *et al.*, 2000). More than 95% of tacrolimus metabolites are eliminated by the biliary route. Urinary excretion accounts for elimination of 2.4% of the drug, on average. Biliary obstruction may increase the concentration of tacrolimus metabolites in the blood (Staatz & Tett, 2004). Animal data indicate that biliary excretion is the main route for elimination of metabolites, 90% (or more) of the administered dose can be eliminated through the excrements (Plosker *et al.*, 2000; Scott *et al.*, 2003).

Side effects

Many side effects are dose dependent and are related to the sites where tacrolimus concentration is high (Shibasaki *et al.*, 2002). The side effects associated with tacrolimus-based immunosuppression are given in Table 1. An overview of the action of tacrolimus on gingiva, bone and cementum are demonstrated.

Tacrolimus and Disturbances in Glucose Metabolism

One of the more serious side effects associated with tacrolimus treatment is the development of a pos-transplantation diabetes mellitus (PTDM), predisposing the patient to complications of diabetes mellitus and to the risk of decreased patient and graft survival (Maes *et al.*, 2001; Scott *et al.*, 2003). The infections are associated with the rapid deterioration of graft function in renal allograft, considering that hyperglycemia and hyperinsulinemia promote atherosclerosis and then, the risk of cardiovascular complications increases. (Drachenberg *et al.*, 1999; Scott *et al.*, 2003).

The influence of diabetes in periodontal tissue is being investigated. It is difficult to get definitive conclusions about the specific effect of diabetes in periodontal tissue, but many alterations are described, including trend to the generalized gingival overgrowth, dental abscesses, periodontitis and dental loss. Perhaps, the principal alteration of non-controlled diabetes was the reduction of the defense mechanism and the increase of the susceptibility to the infections, leading to the destructive periodontal disease (Caldeira *et al.*, 2005) or other oral complications, as showed in Table 2.

The introduction of calcineurin inhibitors associated with the current use of lower doses of steroids reduced the incidence of PTDM (3-14% of patients). However, PTDM remains as an important complication after organ

transplantation (Drachenberg *et al.*, 1999) and seems to be associated to the immunosuppression therapy (Maes *et al.*, 2001).

Direct comparisons can be made between tacrolimus and diabetogenesis. Wakugami *et al.* (1993) realized histological examination and showed that tacrolimus and CsA prevent the reduction in the average size of islets and in the area of β cells in rats, and suggested that the administration of tacrolimus might be a more useful tool to prevent the development of insulin-dependent diabetes mellitus. Drachenberg *et al.* (1999) showed persistent damages in glucose metabolism after pancreas transplantation, and these effects have been attributed to various causes including damages in the insulin secretion and in the systemic drainage of the pancreas. According to the current immunosuppressive regimens employed for pancreas transplants, there are morphological abnormalities of islet cells correlated with drug levels and with damage of glucose metabolism. Tacrolimus showed an increasing in abnormalities in glucose metabolism, observed in patients. The use of pulse steroids with high levels, comparing to tacrolimus, are likely associated with hyperglycemia. The cell damage appears to be reversible and dose dependent.

On the other hand, another field that needs research relates the glucose metabolism disorders as the primary endpoint after the solid organ transplantation. Although all comparisons between calcineurin inhibitors concerning PTDM showed three to five times higher incidence with tacrolimus, many questions remain without answers due to the different criteria used for the diagnosis of diabetes mellitus (based on insulin requirement) or due to the high trough levels of tacrolimus targeted in the initial studies. Data about the mechanism of glucose metabolism disorder in presence of tacrolimus are contradictories (Maes *et al.*, 2001).

Functional abnormalities associated with tacrolimus include decrease in insulin secretion, and more specifically, inhibition of the synthesis of insulin, due to the mRNA transcriptional defect or to the induced hyperinsulinemia that promotes insulin resistance, although in concordance to the animal studies

morphological evidence of islet cell toxicity in pancreas biopsies from patients that received tacrolimus (Van Hoff *et al.*, 1999; Maes *et al.*, 2001). The deleterious effects of calcineurin inhibitors in β cells are morphologically characterized by degranulation, vacuolization, swelling of rough endoplasmatic reticulum, Golgi apparatus, and mitochondria. The association between this class of agents and lipid metabolism abnormalities has been studied and well-documented, but the mechanism remains unclear (Mora, 2005).

Hyperglycemia is a commom metabolic disturb associated with calcineurin inhibitor treatment (Scott *et al.*, 2003). 51% of tacrolimus recipients required a treatment for hyperglycemia at 3 months, reducing to 19% of patients at 1 year follow-up. Notably, with tacrolimus treatment, whereas 47% of these liver transplant recipients had received insulin therapy for 3 months, only 13% of these patients remained in the insulin therapy at 1 year (Scott *et al.*, 2003).

Recently in our laboratory, a gradual time-related progress was observed at longer periods of treatment (180 and 240 days). In those periods, the values of glycemia levels were similar to the control rats. These results are in agreement with Hricik *et al.* (1991) that showed that the toxic damage with tacrolimus may be reversible, and suggested that the abnormalities of glucose metabolism may be slower to normalize after prolonged treatment with this drug. Maes *et al.* (2001) demonstrated the relevant role of the time in the reduction of the glycemia level. Those authors suggested that the abnormalities of glucose metabolism may be normalized after prolonged therapy with tacrolimus.

After long-term administration of Tacrolimus or by an interruption of the drug administration, the disturbance in glucose metabolism would be reversible, but remains unclear by other factors (Maes *et al.*, 2001; Scott *et al.*, 2003; Mora, 2005).

Tacrolimus and Gingival Overgrowth

Gingival overgrowth is characterized by an increase in gingival volume, generally appearing at the interdental papillae and not extending beyond the mucogingival junction. Drug-induced gingival overgrowth is associated with chronic usage of CsA, phenytoin and calcium channel blockers (Ellis *et al.*, 2004). Where gingival overgrowth is associated with important functional or esthetic effects, surgical treatments are possible, but can not prevent recurrence, which often arises because it is impossible to suspend the immunosuppressive, anti-epileptic or anti-arrhythmia treatments (Bader *et al.*, 1998).

Tacrolimus shares many unwanted effects common to other immunosuppressive agents. Many cases reported suggest that the severity of gingival overgrowth observed in patients using tacrolimus is less than that with CsA. The first report detailed a 59-year-old male hepatic transplant recipient changed from CsA to tacrolimus 3 years post-transplant. After 2 months on the new drug regime, the extent of gingival overgrowth was reported to have decreased by 50% to an “acceptable level” (Bader *et al.*, 1998). Further cases support this reduction, and in some cases complete resolution, of gingival overgrowth when changed from CsA to tacrolimus (Hernandez *et al.*, 2000; Thorp *et al.*, 2000; James *et al.*, 2000).

Prevalence studies are limited and the methods used for assessing overgrowth and severity vary from study to study (James *et al.*, 2001; Oettinger-Barak *et al.*, 2001; Wondimu *et al.*, 2001). Few authors do not define a cut-off point at which overgrowth is said to be present, and they do not regard the overgrowth to be clinically significant. Nevertheless, the consensus of the research would suggest that tacrolimus causes less overgrowth than CsA, and when the overgrowth occurs, it is less severe (Ellis *et al.*, 2004). Table 3 showed these studies.

Tacrolimus and CsA have been associated with similar side effects, but contradictory effects despite of the gingival overgrowth. Although it has been reported one case linking FK506 with gingival enlargement (Adams & Famili,

1991), most clinical trials that compare the effectiveness of tacrolimus and CsA after cadaveric organ transplants tend to suggest that patients treated with tacrolimus infrequently complain of gingival problems (James *et al.*, 2001). Three patients have been observed for 12 months (Adams & Famili, 1991), but one of these exhibited no hyperplasia for the first 9 months posttransplant, but hyperplastic changes were evident when intraoral records were made at 12 months. This patient began with nifedipine 1 month posttransplant and Procardia XL at month 8 posttransplant. The etiology of this hyperplasia is unclear.

In other study, Oettinger-Barak *et al.* (2001) showed that patients treated with tacrolimus manifested greater gingival overgrowth than compared to the controls, although significantly lower than CsA-treated patients. The authors compared this overgrowth with the experimental group and they observed that it was not significantly different. This fact suggests that this overgrowth could be primarily attributed to the past liver disease and to the concomitant edema associated with it, with little additional effect of tacrolimus.

Other studies demonstrate that tacrolimus has no adverse effects on the gingival tissues, thus it has potential like an alternative immunosuppressant for individuals susceptible to develop CsA-induced gingival overgrowth (Bader *et al.*, 1998; James *et al.*, 2001), probably because tacrolimus is a potent anti-inflammatory and its action causes a decrease in the exudates formation and decreases polimorfonucleaters and monocytes (Singh *et al.*, 2003) or may suppress TNFα, IL-1β and IL-6 cytokines inflammatory (Miyata *et al.*, 2005).

In a preliminary study, James *et al.* (2000) presented conclusive results and showed that the conversion to tacrolimus may be beneficial for suitable patients with marked gingival overgrowth related to immunosuppressive therapy with CsA. However, in the short-term at least, while the use of tacrolimus may be associated with substantial reduction in the severity of gingival overgrowth, it may only be in a minority of cases that there will be almost complete regression, according to Hernandez *et al.* (2000), Thorp *et al.* (2000) and Spolidorio *et al.* (2005), but contradictory results of Ellis *et al.* (2004) when patients who have had

alteration of their immunosuppressant from CsA to tacrolimus may persist demonstrated gingival overgrowth, which may be attributed to their ongoing calcium channel blocker therapy. The effective plaque dental control must be done and it does not depend of the maintenance of the gingival overgrowth and conversion from CsA to tacrolimus.

Tacrolimus and Bone Disease

Bone loss following transplantation is usually rapid in the early phase with stabilization after 1 year, although this general statement conceals the reality of wide variability between apparently similar patients and also between the different categories of organ recipients. Fracture rates are variable and often extremely high. Compared with expected rates in the normal population, fracture incidence was five times higher in male kidney recipients aged 25 to 64 years. In women, the incidences were 18 and 34-fold higher in kidney recipients aged 25-44 years and 45-64 years, respectively. The high fracture rate saw in dialysis patients increases even after successful kidney transplantation, especially in diabetics. Bone loss rate following cardiac, liver and lung transplantation is strikingly high during the first 6 months with fracture rates match, ranging from 22% to 36%, 24% to 65% the age bands that received cardiac transplants and experimented fracture incidence 13-fold higher than expected (Cunningham, 2005)

As indicated above, the impact of the posttransplantation environment is conditioned to a substantial degree by the type and severity of pretransplant morbidity. They experiment an exceptionally high fracture rate after transplantation – not surprising given that following transplantation an already poor quality skeleton is bombarded with negative influences, many of them iatrogenic (Fig. 3) (Cunningham, 2005).

The calcineurin inhibitors, CsA and tacrolimus, have complex and incompletely understood actions on bone. On the plus side, both have reduced dramatically the requirement for prolonged high dose of glucocorticoids following

transplantation, although in most cases have not eliminated this requirement completely, albeit the principal impact of CsA, but not necessarily tacrolimus, at least experimentally and also clinically possibly, is to accelerate bone resorption (Fig. 4) (Cunningham, 2005).

Tacrolimus therapy has been shown in some clinical reports to cause bone mineral loss as severe as that caused by CsA (Cvetkovic *et al.*, 1994; Park *et al.*, 1997; Cayco *et al.*, 2000; Stempfle *et al.*, 2002). In other studies tacrolimus does not appear to induce severe osteopenia by high-turnover bone metabolism in rat (Inoue *et al.*, 2000) or in patients (Goffin *et al.*, 2002; Goffin *et al.*, 2003). In most cases, however, tacrolimus is being used in combination with steroids including prednisolone, and according to the effect of tacrolimus alone on bone mineral metabolisms it could not be accurately elucidate (Cunningham, 2005).

Cvetkovic *et al.* (1994) showed, with histomorphometry indices, that tacrolimus causes accelerated bone remodeling, with resorption far in excess of formation, leading to a loss of trabecular bone volume, albeit with elevated doses at tacrolimus.

Although the molecular mechanisms for the immunosuppression caused by CsA and tacrolimus have been extensively investigated, the precise mechanisms of the immunosuppressant-induced high-turnover osteopenia are still unclear, and there are several possibilities. First, the agents may have a direct toxic effect on bone cells (osteoclasts). Second, the agents may act directly on bone cells and affect their ability to secrete local autocrine factors or respond to systemic hormones. Finally, the agents may have an indirect action on bone via their effects on the immune system, decreasing cytokine/lymphokine production. In all probabilities, there is a combination of the latter two explanations that underlies the effects of the immunosuppressant on bone (Cvetkovic *et al.*, 1994; Stempfle *et al.*, 2002).

Factors other than corticosteroids must influence osteopenia. Both CsA and tacrolimus have been shown to cause osteopenia in experimental animals. The contribution of CsA and tacrolimus is difficult to estimate here due to the

differences in steroid dose between the two drugs. However, no benefit of tacrolimus immunosuppression was seen over that of CsA. It may be that both or neither of these drugs have a major etiologic role in the production of osteopenia, considering that their effects are comparable. Tacrolimus may be more deleterious to bone (an effect which counteracts the effect of reduced steroids), or may increase renal dysfunction, and this fact may exacerbate the osteopenia (Park *et al.*, 1996).

The role of tacrolimus on the skeleton is not well understood. In one ex0perimental rat model, tacrolimus caused histologically an increase in bone formation and especially bone resorption, leading to a significant loss of trabecular bone volume that was even more pronounced than that observed with CsA. In contrast to CsA, effects of tacrolimus on biochemical markers include a decrease of ionized calcium, accompanied by an increase in parathyroid hormone (PTH) and no increase in $1,25(OH)_2D$ (calcitriol) and osteocalcin after administration (Stempfle *et al.*, 2002). However, high dose tacrolimus-based immunosuppressive regimen is associated with a rapid bone loss early after cardiac transplantation. Beyond the first 6 months after transplantation, calcium vitamin D and hormone supplementation in hypogonadism level sufficiently lead to bone mineral recovery, and low dose calcitriol should be substituted for at least 2 years as additional antiresorptive therapy (Stempfle *et al.*, 2002).

In contract with others studies, tacrolimus did not affect the excretion of urinary dexypyridinoline, a marker of bone resorption, whereas CsA increased it. The results of the study of Inoue *et al.* (2000) clearly demonstrate that, in comparison with CsA, tacrolimus did not cause severe bone loss. Thus, considering that tacrolimus causes less reduction in bone mineral density compared with CsA in rats, it can be expected that the tacrolimus-treated patients might show a lower incidence of fracturing compared with CsA (Inoue *et al.*, 2000).

Another recent study has shown that tacrolimus has the potential to elevate plasma and hepatic levels of insulin-like growth factor (IGF)-I in rats, but

it was not observed for CsA (Epstein *et al.,* 1995). IGF-I is the most potent of IGFs and has potent osteogenetic properties on bone metabolism, evoking the idea that tacrolimus, unlike CsA, might have beneficial effects on bone metabolism (Epstein *et al.*, 1995; Inoue *et al.*, 2000).

There are no previous data comparing the effect on alveolar bone height of CsA and tacrolimus, and there are no data regarding the effect of tacrolimus itself on alveolar bone height. In the study of Oettinger-Barak *et al.* (2002) with patients and radiographic analysis, there were no statistically significant differences in alveolar bone height between CsA and tacrolimus.

Tacrolimus might exert a direct trigger effect on bone cells, affecting the osteoblasts more than the osteoclasts (thus, counteracting the effects of glucocorticoid), or an indirect effect through cytokines that affects bone cells. In this context, Inoue *et al.* (2000) recently showed that rats treated with tacrolimus at a dose used to prevent allograft rejection had no significant reduction in their bone mass. In addition, Yoshikawa *et al.* (2000) have reported that allogeneic cultured bone constructs implanted in rats treated with tacrolimus exhibited better bone formation than those implanted in control rats (Goffin *et al.*, 2002).

The potential advantage of tacrolimus over CsA on bone mineral density could be explained either by a better induction of IGF-1, a powerful activator of osteoblastic function, a more “bone-friendly” expression of the different T lymphocyte subclasses (Voggenreiter *et al.*, 2000), or by an indirect effect through cytokines that affects bone cells (Goffin *et al.*, 2003).

Conclusion

It may be concluded from the results that the conversion to tacrolimus may be beneficial for suitable patients with marked gingival overgrowth, comparing to the immunosuppressive therapy with CsA.

Data presented in the literature about the action of tacrolimus on the bone tissues are uncertain and conflicting, because there are not data regarding the

effect of tacrolimus itself on alveolar bone height. But tacrolimus might exert a direct trigger effect on bone cells, affecting the osteoblasts more than the osteoclasts, or this drug may have an indirect effect through cytokines and affects bone cells.

However, diverse studies must be done to attempt and to establish the real action of tacrolimus on periodontal tissue, special on the bone, virtue of the appropriated dose, method of administration and period of treatment, thus showing its true osteopenic action.

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Table 1: The side effects associated with tacrolimus based immunosuppression.

Side effects	***Author(s)***
Gingival overgrowth	Adams and Famili , 1991 Oettinger-Barak *et al.*, 2001 Ellis *et al.,*2004
Hirsutism	Kari and Trompeter, 2004
Pruritus	Becker *et al.*, 2006
Neurotoxicity	Corruble *et al.*, 2005 Umapathi and Chaudhri, 2005
Nephrotoxicity	Corruble *et al.*, 2005 Tamada *et al.*, 2006
Abnormalities bone metabolism	Scott *et al.*, 2003 Staatz & Tett, 2004
Hypertension	Crespo-Leiro, 2005
Alopecia	Tricot *et al.*, 2005
Risk of infections (viral, bacterial and fungal)	Spencer *et al.*, 1997 Plosker *et al.*, 2000
Diarrhea	Vitko *et al.*, 2005
Diabetogenesis (disturbances in glucose metabolism)	Marchetti & Navalesi, 2000 Baltar *et al.*, 2005
Malignancies (particularly lymphoma and malignancy of skin)	Scott *et al.*, 2003
Gastrintestinal disturbances	Staatz & Tett, 2004
Hyperkalaemia	Staatz & Tett, 2004
Hypomagnesaemia	Staatz & Tett, 2004
Serum lipids and lipoprotein changes	Staatz & Tett, 2004

Table 2: The side effects associated with diabetes mellitus and oral manifestations.

Side effects	***Author(s)***
Cheilosis	Wermers *et al.,* 1996
Burning mucosa and língua	Costa *et al.,* 2004
Dental caries	Siudikiene *et al.*, 2005
Oral candidiasis	Kumar *et al.*, 2005 Costa *et al.,* 2004
Xerostomia	Costa *et al.,* 2004
Gingivitis	Chapper *et al.*, 2005 Salvi *et al.,* 2005
Periodontitis	Torrungruang *et al.*, 2005 Khader *et al.,* 2006
Clinical attachment loss	Torrungruang *et al.*, 2005 Khader *et al.,* 2006
Dental abscesses	Caldeira *et al.*, 2005

Table 3: The studies of prevalence of gingival overgrowth or no associated with tacrolimus based immunosuppression.

Drugs	***Prevalence of gingival overgrowth***	***Author(s)***
Tacrolimus	Positive	Adams and Famili, 1991 Basile *et al.*, 1998 Oettinger-Barak *et al.*, 2001 Ellis *et al.*, 2004
Tacrolimus	Negative	Hernandez *et al.*, 2000 Thorp *et al.*, 2000 James *et al.*, 2001
Conversion from CsA to tacrolimus	Negative	Bader *et al.*, 1998 Kohnle *et al.*, 1998 James *et al.*, 2000 Spolidorio *et al.*, 2005

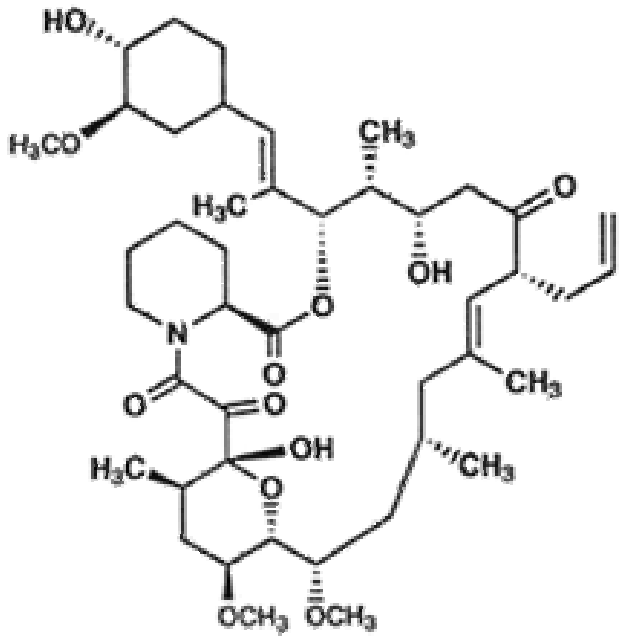

Fig. 1: The Chemical Structure of Tacrolimus (FK506) - The Pharmaceutical Society of Japan, 2004

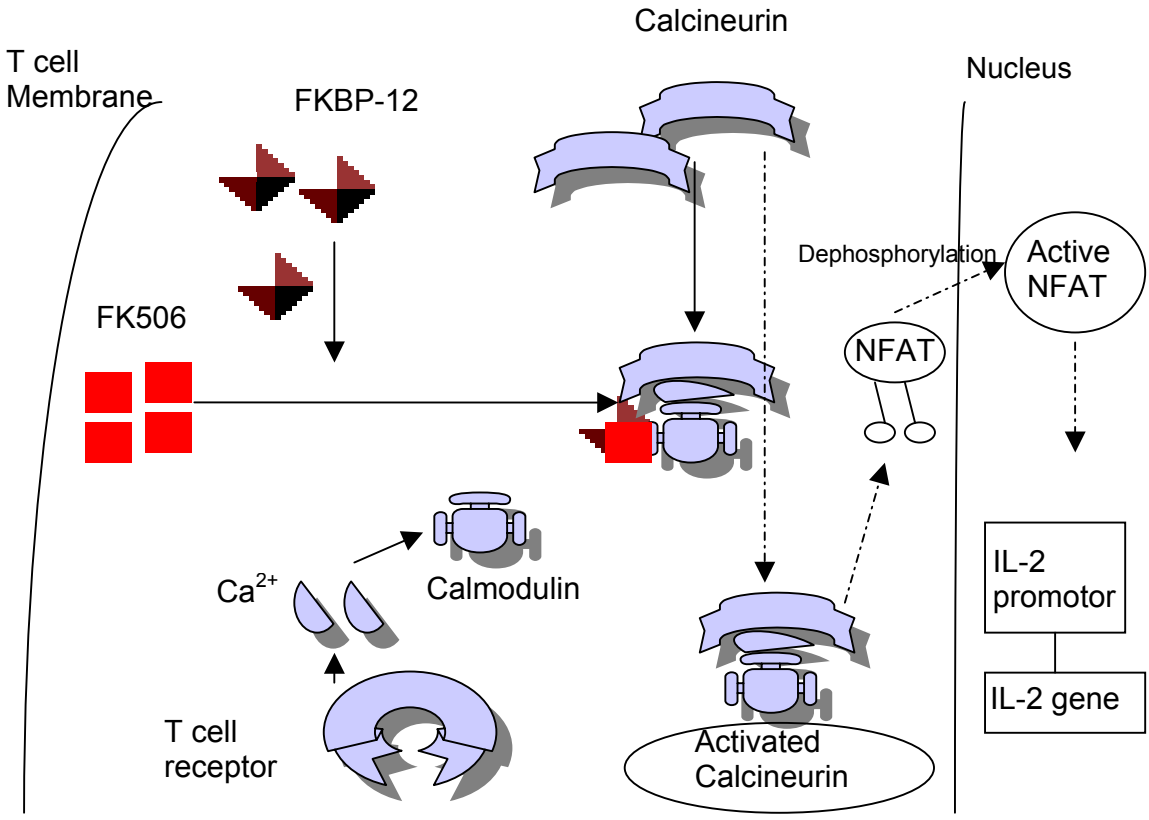

Fig. 2: Immunophilin-mediated inhibition of T cell activation. Stimulation of the T cell receptor complex results in an inositol 1,4,5-triphosphate-mediated increase in intracellular calcium level resulting in IL-2 gene transcription. FK506 inhibit this process by binding their respective immunophilin, FKBP12, to inhibit the calcium/calmodulin-dependent protein phosphatase, calcineurin. FKBP-12 = FK506-binding protein; NFAT nuclear factor of activated T cells.

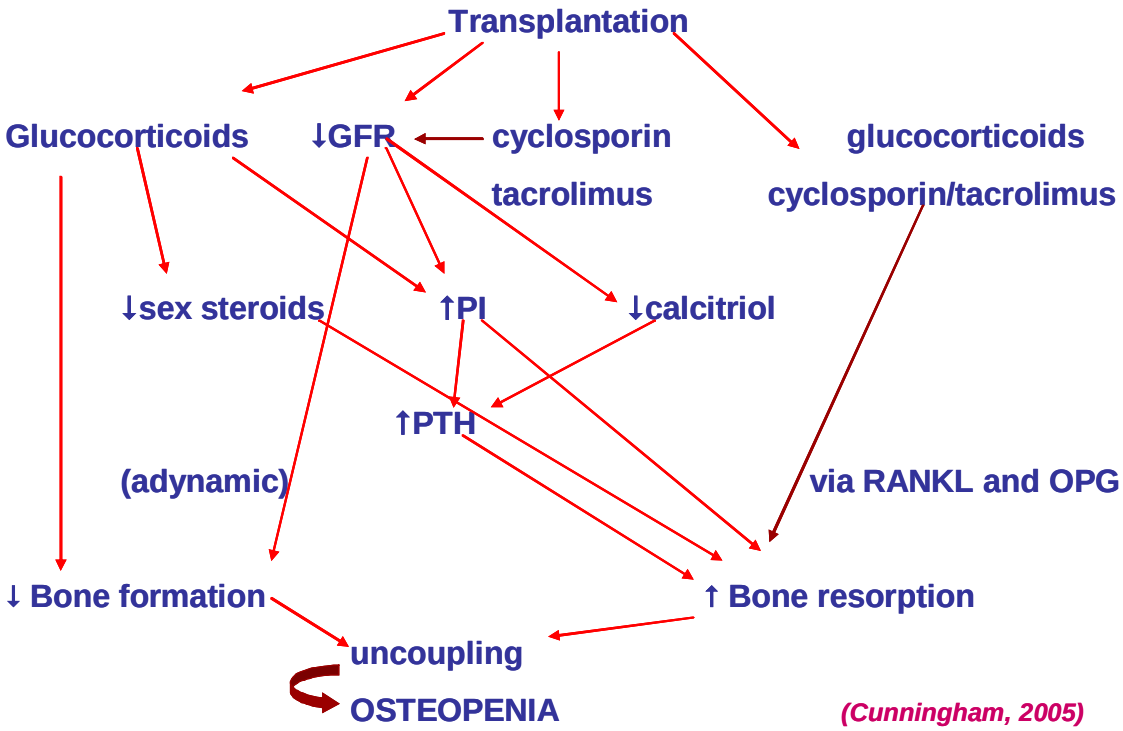

Fig. 3: Impact of the posttransplant environment on the skeleton. The scenario during the early posttransplant phase is depicted. The adverse influences are dominated by glucocorticoids and calcineurin inhibitors, and in the case of kidney transplantation, reduced glomerular filtrate rate (GFR). PI, inorganic phosphate; PTH, parathyroid hormone; RANKL, receptor activator of NFKbeta ligand; OPG, osteoprotegerin (extracted of Cunningham, 2005).

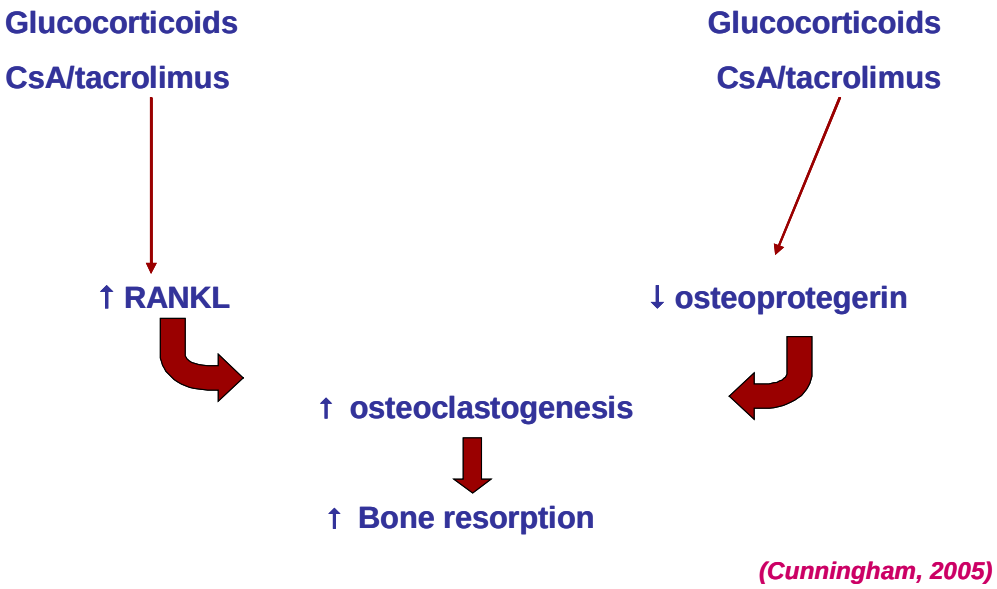

Fig. 4: Proposed role of RANKL (receptor activator of NFKbeta ligand) and osteoprotegerin in the mediation of glucocorticoid and calcineurin inhibitor toxicity (extracted of Cunningham, 2005).

EFFECTS OF LONG-TERM FK506 THERAPY ON THE GINGIVAL TISSUES OF RATS – A MORPHOLOGICAL EVALUATION.

Submetido à publicação no periódico Journal of Periodontal Research.

Effects of long-term FK506 therapy on the gingival tissues of rats - A morphological evaluation.

Carlos Augusto Nassar, Patricia Oehlmeyer Nassar, Denise Carleto Andia, Morgana Rodrigues Guimarães, Luis Carlos Spolidorio

Abstract

Background: Tacrolimus (FK506) is an immunosuppressive drug in organ transplantation. It is suggested that FK506 is not induced gingival overgrowth. Nevertheless there are not studies exploring the effects of long-term FK506 therapy on gingival tissues.

Objective: The purpose of this study was to evaluate the effects of long-term therapy with FK506 on the gingival tissue.

Material and methods: Rats were treated for 60, 120, 180 and 240 days with daily subcutaneous injection of 1mg/Kg body weight of FK506. After histological processing, the oral and connective tissue, as well as, volume densities of fibroblasts (Vf), collagen fibers (Vcf) and others structures (Vo) were assessed at the region of the lower first molar.

Results: After 60 and 120 days of treatment with FK506 not observed gingival alterations. After 180 and 240 days of treatment with FK506, evident gingival overgrowth associated a significant increase of epithelium and connective tissue was observed. There was an increase of Vf, Vcf and Vo.

Conclusions: Within the limits of this experimental study, it can be concluded that FK506 induced increase of fibroblast and collagen tissue in parallel with the severity of the overgrowth after long-term of treatment.

Key words: tacrolimus; gingival overgrowth; immunosuppression drugs; animal study

Introduction

Tacrolimus (formerly known as FK506) was introduced as an immunosuppressive agent for use in organ transplants in 1987 and has gradually been gaining popularity. The pharmacodynamics of FK506 is very similar to cyclosporin A (CsA) and is a potent immunosuppressive agent used as an alternative to CsA to prevent graft rejection and to treatment autoimmune diseases (1, 2). FK506, a macrolide immunosuppressant, inhibits cellular and humoral immune responses via several mechanisms of action, with the central effect involving inhibition of calcineurin. Inactivation of calcineurin via complex formation with the immunophilin FK506 binding protein 12 (FKBP12) prevents the translocation of the transcription factor nuclear of activated T cells that promotes interleukin-2 (IL-2) mediated proliferation of helper T cells (1, 2). It has been used successfully to prevent renal, liver and cardiac allograft rejection, although it can cause side effects such as nephrotoxicity, neurotoxicity and glucose metabolism disorders. On the other hand, hyperlipidaemia, hypertension and hirsutism are less likely with FK506 than CsA (2). Unlike CsA, however, FK506 does not appear to induce gingival overgrowth (3, 4), albeit the some authors suggest, in a number of cases reports, that the severity of gingival overgrowth seen in patients taking FK506 is less than that with CsA (5, 6, 7).

Prevalence studies are limited and the methods used for assessing overgrowth and severity varies from study to study (8, 9). Few authors define a cut-off point at which overgrowth is said to be present, but nor do they regard the overgrowth to be clinically significant. Nevertheless, the consensus of the research thus far would suggest that FK506 causes less overgrowth than CsA, and that when overgrowth does occur it is less severe (10),

Up to now, clinical trials have only shown that FK506 treatment is not commonly associated with the development of gingival overgrowth. However, further observations are necessary in human and experimental models. The purpose of this study was to describe the histometry and densities of fibroblasts, collagen fibers and the others structures in the gingival tissue of rats that treated with FK506 for long-term.

Materials and methods

Eighty male Holtzman rats (*Rattus novergicus albinus*) weighing 50g were housed under similar conditions in cages with access to food and water *ad libitum*. The animals were randomly distributed in eight groups of 10 animals each. All protocols described below were approved by the Institutional Experimentation Committee of the School of Dentistry of Araraquara, Araraquara, São Paulo, Brazil. Four groups were treated with FK-506 (Prograf® - Janssen Cilag, Brazil) injected subcutaneously in a daily dose of 1mg/Kg body weight (11, 12). Four groups were used as controls and received subcutaneous injection of saline solution during all periods. The experimental periods were 60, 120, 180 and 240 days. According to others authors, this dosage provides plasma peak and trough levels of FK506 of 11.2 ng/mL approximately (13, 14, 15). Control rats from the other from groups were daily injected subcutaneously with saline (NaCl 0.9%). All rats were weighed weekly.

HISTOLOGY TECHNIQUES

The rats were killed by an overdose of anesthesia (Ketamine - Francotar®, Virbac do Brazil Ind. e Com. Ltda, São Paulo, São Paulo, Brazil) at the end of experimental periods and the mandibles were carefully removed, and soaked in 10% formalin. Decalcification was carried out in solution of Morse (50 mL of 50% formic acid and 50 mL of 20% sodium citrate). Five micrometers (5μm) serial paraffin sections were made on the bucco-lingual aspects of the whole 1st left and right lower molar and stained with hematoxylin and eosin. Each 1st lower molar has a mesial-distal diameter of approximately 1mm, producing sections of 5 μm each. Histometric and stereological studies were made on the buccal gingiva.

HISTOMETRY

Gingival epithelium and connective tissue area measurements were made with the help of a Zeiss microscope at a magnification of 125x using a Sigma computer program (Mocha, Jandel Scientific, CA, San Rafael, USA). From each tooth, were made 10 measurements in sections of 50-μm intervals each. For

statistical analysis the mean from each animal was used, calculated from the 20 measurements obtained from the 1st right and left molars (Fig. 1).

STEREOLOGY

Volume densities of fibroblasts (Vf), collagen fibers (Vcf) and others structures (Vo), i.e. blood vessels, nerves and unidentified structures, were estimated according to the principles established by Dellesse (16), which were applied to histology by Weibel (17). The count was performed with the help of a Zeiss microscope, using oil immersion at a magnification of 1000X. A square lattice of 25 test points was projected into the microscope ocular, with the use of microvid system that connected the microscope to a computer. For each animal, 16 sections were selected (eight from the left molar and eight from the right), and 25 points were counted in each section. Vf, Vcf and Vo were expressed as percentages of the total points counted.

STATISTICAL ANALYSIS

Measurements were expressed as mean and standard deviation. Statistical analyses were made by 1-way analysis of variance (ANOVA) and Tuckey-test.

Results

HISTOLOGIC

The gingival of the control rats as well as of the rats treated with FK506 for 60 and 120 days, showed normal morphology in all analyzed periods, showed keratinized stratified squamous epithelium. The interface between epithelium and connective tissue was strongly interdigitated: many tall, narrow connective tissue papillae project into the epithelium. Alternating with these is usually as taller, thin epithelial extension, projecting the underlying connective tissue. The connective tissue was dense, and showed fine collagen fibers that were interspersed with delicate vessels and fibroblasts. After 180 and 240 days, gingival overgrowth was observed in all gingival areas, but it was more evident on the buccal gingival tissue of the lower molar teeth. The gingival epithelium was hyperplastic, with

deep papilla interdigitations. The connective tissue was dense, and showed thick collagen fibers that were interspersed with delicate vessels and fibroblasts. It was observed little inflammatory cells.

HISTOMETRIC FINDINGS

Table 1 show the linear measurements (μm±SD) of the epithelium and connective tissue of the buccal gingiva for the 1st lower first molars of control and treated rats. The gingival of the control rats as well as of than FK506-treated rats for 60 and 120 days were similar ($p>0.05$). On the other hand, the linear values of the FK506-treated rats were significantly increased at 180 and 240 days of treatment compared with control as well as at 60 and 120 days ($p<0.05$).

STEREOMETRIC FINDINGS

The stereometric findings of the control group and of the groups treated with FK506 are demonstrated in Table 2. In the control groups, the volumetric densities of fibroblasts, collagen fibers and other structures were 11.87%, 66.66% and 21.47% in the buccal gingiva and stayed constant in all the studied periods. After 60 and 120 days of the treatment the values of Vf, Vcf and Vo were similar to the control group ($p>0.05$). There was a significant increase of Vf and Vcf at 180 and 240 days ($p<0.05$), while the volumetric densities of other structures decreased when compared of the control groups ($p<0.05$).

Discussion

The present study evaluated the gingival overgrowth following long-term administration of FK-506 in rats. Some studies have shown that FK506 not induced gingival overgrowth (3, 4) or suggested that the severity of gingival overgrowth seen in patients taking FK506 is less than that with cyclosporine (6, 18). For our knowledge, there are not available data evaluating the effects of long-term therapy with FK506 on the gingival tissue in rats, however, we investigated the effect of a systemic treatment of rats with the

immunosuppressant FK506 for long-term on histological, histometric and stereometric analysis.

In this study, the use of a rat model allowed the strict control of some variables, such a genetic predisposition, gender and dose. In addition, the chosen dose 1 mg/Kg body weight was sufficient to achieve therapeutic FK506 serum levels (11, 12, 19), recently in our laboratory verified that serum levels of FK506 (data not publishing). This dose is clinically relevant and within the range of doses used in studies on organ and limb transplantation that usually are between 0.6 and 1.0 mg/Kg body weight (13, 14, 15, 20, 21), resulting in consistent responses.

In agreement with previous studies (5, 6), this work showed that FK506 administration for brief periods of treatment (60 and 120 days) not induced gingival overgrowth. Interestingly, in the present study, gingival overgrowth was observed at longer periods of treatment (180 and 240 days) in all rats. The gingival overgrowth was more evident on the buccal gingival tissue of the lower molar teeth. The gingival epithelium was hyperplastic, with deep papilla interdigitations, with the connective tissue increased and dense, when compared with respective control groups.

It was recently verified that after briefer periods of treatment with CsA, there was evident gingival overgrowth associated with a significant increase of epithelium and connective tissue. After 180 and 240 days of the treatment, there was a reduction of the gingival overgrowth with significant decreases of epithelium and connective tissue (22). These results are in agreement with a prospective longitudinal study (23) that showed the relevant role of time in reducing gingival overgrowth in heart transplanted patients undergoing CsA therapy from 6 to 18 months after transplantation. A time-dependent pattern of gingival overgrowth in nifedipine-treated animals was also demonstrated by Fu *et al*. (24). Therefore FK506 not present the same effect on the gingival tissue.

The exact pathogenesis of FK506-induced gingival overgrowth after long period of treatment is not known. We have been to speculate that the gingival

overgrowth could be the result of a gradual sensibilization of the gingival fibroblasts as well as of gingival epithelium. After long-term of FK506 therapy can have a action direct or indirect on determined population of gingival fibroblasts and collagen fibers via cytokine and growth factors. Fibroblasts heterogeneity remains one of the key factors used to explain the variable response of the gingival tissue to the various drugs (25).

Several *in vivo* and *in vitro* studies have investigated the changes in tissue composition and cellular function that accompany drug-induced gingival overgrowth, mainly those induced by CsA or calcium channel blockers, most of the attention to date has focused on cytokine expression alterations (26, 27, 28).

In line with the present work, Frizell *et al*. (29) verified that FK506 increases hepatic collagen and increase RNA levels of transforming growth factor beta 1 and collagens I, III and IV, reducing hepatic fibrosis after 240 days of treatment. Some cytokine is a multifunctional peptide that regulates diverse biologic activities including cell growth, cell death or apoptosis, cell differentiation, and extracellular matrix synthesis (30).

Gagliano *et al*. (31) found that FK506 strongly induced matrix metalloproteinases (MMP)-1 gene expression, and the pattern was similar for, MMP-1 collagenolytic levels in fibroblasts after FK506. By contrast, MMP-2mRNA levels increased 48 and 72 h after FK506 treatment, while gelatinase protein levels seemed unaffected by FK506. In the same fibroblast samples they evaluated the effect of CsA on MMP levels, and found that CsA lowered MMP-1 in the supernatants of CsA treated fibroblasts (32). This confirms that MMP-1 has a key role in the mechanisms of gingival overgrowth development.

In the present work suggest that special attention should be given to the clarification of the mechanisms of action of FK506 on the index fibroblasts proliferation and collagen fibers *in vitro* and *in vivo* as well as in the protein synthesis and colagenolytic activity, mainly with longer periods of treatment with FK506.

Nevertheless, detailed studies are still needed to clarify the possible cellular and molecular mechanisms involved in the effect of the FK506 on the gingiva after long-term administration.

Within the limits of this experimental study, it can be concluded that FK506 induced increase of fibroblast and collagen tissue in parallel with the severity of the overgrowth after long-term of treatment.

Acknowledgements

We are especially grateful to José Antônio Sampaio Zuanon for the carefully histological processing and FAPESP for the financial support.

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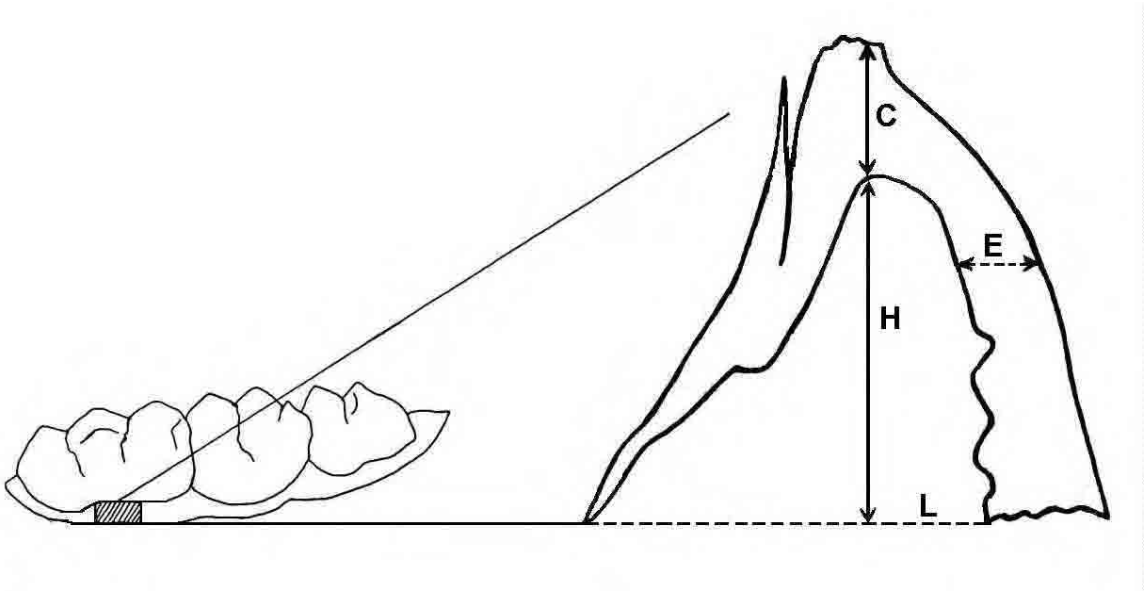

Fig. 1- Schematic illustration showing the regions where linear measurements were made. *E*, epithelium thickness; *C*, epithelium crest; *H*, gingival connective tissue height; *L*, connective tissue width

Table 1 – Linear measurements (μm± SD) of epithelium thickness (*E*), epithelium crest (*C*), gingival connective tissue height (*H*) and connective tissue width (*L*) of the buccal gingival of first lower molars of normal rats and treated with FK506 in various periods of treatment.

		PERIODS			
	TREATMENT	**60d**	**120d**	**180d**	**240d**
E	Control	45.8±0.6	43.3± 0.4	48.5±1.4	44.6±0.8
	FK506	45.7±1.9 [a]	43.4±2.0 [a]	67.5±0.2 [b]*	87.0±0.3 [c]*
C	Control	46.9±0.4	41.3± 0.7	45.7±1.8	47.7±0.7
	FK506	46.4±2.1 [a]*	41.2±1.5 [b]	69.8±0.2 [c]*	69.0±0.2 [c]*
H	Control	415.9±1.1	414.9± 1.2	418.8±0.7	409.7±1.8
	FK506	415.7±0.9 [a]	413.8±0.5 [b]*	493.8± 0.7[c]*	570.8±0.8 [d]*
L	Control	141.9± 1.5	147.4± 0.9	166.1 ± 2.3	139.0±0.5
	FK506	139.7±0.5 [a]*	145.5 ± 0.7 [b]*	218.5± 0.5 [c]*	260.5±0.6 [d]*

Different letters represent statistically significant difference among means in the same group. *p<0.05, statistical significance vs. control rats in the same period.

Table 2 – Volumetric densities of fibroblasts (Vf), collagen fibers (Vcf) and others structures (Vo) from the buccal gingival region of the first lower molar in control and FK506 rats. Values present means ± SEM. The results are expressed as percentages.

		PERIODS			
	TREATMENT	**60d**	**120d**	**180d**	**240d**
Vf	Control	11.87±0.3	11.01±0.2	10.93±0.4	10.81±0.2
	FK506	11.80± 1.0 [a]	11.40± 1.1 [b]*	12.40±1.1[c]*	12.80±1.2[d]*
Vcf	Control	66.66±0.2	66.70±0.3	65.90±0.2	68.68±0.3
	FK506	66.76±1.8 [a]	66.18±2.3 [a]	69.00±1.8[b]*	73.2±1.9 [c]*
Vo	Control	21.47±0.6	22.29±0.3	20.17±0.3	20.51±0.2
	FK506	21.44±1.5 [a]	21.80±1.9 [a]	18.60±1.4[b]*	14.00±1.5 [c]*

Different letters represent statistically significant difference among means in the same group. *p<0.05, statistical significance vs. control rats in the same period

THE EFFECTS OF LONG-TERM FK506 THERAPY ON THE ALVEOLAR BONE AND CEMENTUM OF RATS.

Submetido à publicação no periódico Journal of Periodontal Research.

The effects of long-term FK506 therapy on the alveolar bone and cementum of rats.

Carlos Augusto Nassar, Patricia Oehlmeyer Nassar, Denise Carleto Andia, Morgana Rodrigues Guimarães, Luis Carlos Spolidorio

Abstract

Background: The calcineurin inhibitors, cyclosporine A (CsA) and tacrolimus (FK506) have complex and incompletely understood actions on bone. FK506 is an alternative therapy used for transplant patients with refractory graft rejection or those intolerant to CsA, without the adverse effects frequently attributed to CsA therapy; however bone loss following transplantation is usually rapid in the early phase of the transplant.

Objectives: The objectives of the present study were to evaluate the effects of long-term therapy with tacrolimus on the alveolar bone metabolism and cementum.

Material and methods: Rats were treated for 60, 120, 180 and 240 days with daily subcutaneous injection of 1mg/Kg body weight of FK506. After period experimental, blood collection was obtained and serum levels calcium and alkaline phosphatase (ALP) were measured. After histological processing, the alveolar bone and cementum, as well as, volume densities of bone (V_b) and osteoclasts (V_o) were assessed at the region of the lower first molar.

Results: There was a tendency towards a statistically significant decrease in ALP levels with FK506; however serum calcium levels increased during long periods when compared with the control group. After 60, 180 and 240 days of treatment with FK506 not observed V_b and V_o alterations. After 120 days of treatment, evident decrease V_b associated a significant V_o was observed, but it was not showed alveolar bone loss. Not observed any alterations of cementum in rats treated with FK506.

Conclusion: So, it may be concluded that FK506 administration has not induced side effects on the periodontium.
Key-words: alkaline phosphatase; calcium; FK506; alveolar bone; cementum

Introduction

Progressive bone loss after transplantation is one of the side effects after transplantation. Several lines of evidence have suggested that preexisting bone diseases immunosuppressive drugs (1). Cyclosporin A (CsA) and tacrolimus (FK506) are the most commonly used immunosuppressant to reduce the rejection of allogeneic organ transplant. Although structurally unrelated CsA and FK506 have similar cellular effects on T-cell activation and the lymphokine-monokine cascade (2, 3).

FK506 is an alternative therapy used for transplant patients with refractory graft rejection or those intolerant to CsA, without the adverse effects frequently attributed to CsA therapy; however bone loss following transplantation is usually rapid in the early phase of the transplant (4).

Experimentally have suggested that CsA accelerates bone resorption and leads quickly to a severe high turnover osteopenic state, albeit some studies demonstrated that CsA decrease bone resorption and increased bone formation in rats (5), and in alveolar bone also decrease bone resorption with imbalance in the metabolism bone (6). However, a severe osteopenia responsible for osteoporotic fractures in transplantation recipients has been described by some authors (7,8). Furthermore, an increased osteoclasia and decreased bone formation at periodontal sites have been observed in the alveolar bone of rats treated with CsA (9). On the other hand suggested that systemic CsA administration induced the formation of new cementum in rat molar, and they are not reversible after cessation of therapy (10).

The action of FK506 on cementum was not still accounted and in the bone, *in vitro,* is still uncertain and experimental data obtained from animal models suggests that FK506 is an osteopenic agent (8). However, more recent

studies suggest that FK506 is less osteotoxic than CsA (11,12). In contrast to CsA, effects of FK506 on biochemical markers include a decrease in ionized calcium, accompanied by an increase in parathyroid hormone (PTH) and no increase in $1,25(OH)_2D$ (calcitriol) and osteocalcin after administration (8,13).

In general, long-term administration of immunosuppressant at high doses induces high-turnover osteopenia. In a previous study, however, Yoshikawa *et al* (14) reported that low-dose and short-term application of FK506 promoted bone formation in cultured allogeneic and isogeneic rat bone grafts. Indeed, they showed that FK506 had an excellent effect on bone formation; however, they used osteoconductive porous hydroxyapatite with the bone grafts, thus, the effect of FK506 on osteoinduction in the absence of osteoconduction remains to be clarified (15).

The process of bone formation consists of differentiation of the mesenchymal cells into osteoblasts, osteoblasts proliferation, bone matrix deposition and mineralization. Enhanced expression of alkaline phosphatases (ALP) occurs at the maturation stage of osteoblastic differentiation, and an increase in osteocalcin expression occurs at the mineralization stage (16), although serum calcium showed correlation with bone formation, however, this may decrease with kidney disease for long-term (1). Therefore, the objectives of the present study were to evaluate the effects of long-term therapy with tacrolimus on the alveolar bone metabolism and cementum.

Material and methods

Animals

Eighty male Holtzman rats (*Rattus novergicus Albinus*) weighing 50g were housed under similar conditions in cages with access to food and water *ad libitum*. The animals were randomly distributed in eight groups of 10 animals each. All protocols described below were approved by the Institutional Experimentation Committee of the School of Dentistry of Araraquara, Araraquara, São Paulo, Brazil. Four groups were treated with FK506 (Prograf® - Janssen Cilag, Brazil) injected subcutaneously in a daily dose of 1mg/Kg body

weight (17,18). Four groups were used as controls and received subcutaneous injection of saline solution during all periods. The experimental periods were 60, 120, 180 and 240 days. The rats were weighed weekly and monitored for abnormal appearance of coat and abnormal activity levels.

Calcium and alkaline phosphatase analysis

At the end of experimental periods, the rats were anesthetized with 0.08mg/100g body weight Ketamine (Francotar®, Virbac do Brazil Ind. e Com. Ltda, São Paulo, São Paulo, Brazil) and 4-5ml of blood was obtained by direct cardiac puncture in heparinized capillary tubes for immediate calcium measurements, using an ICA-1 ionized calcium analyzer (Radiometer Company, Copenhagen, Denmark); other blood samples were centrifuged and the serum stored at -70°C until assay. Total serum alkaline phosphatase activity was measured colorimetrically (ALP Kit-Sera Pak, Bayer AG, Elitech, France) using paranitrophenyl phosphate as the substrate. Alkaline phosphatase activity was measured by the absorbance at 405 nm, using Technicon SMA-24 (Technicon, Domont, France). The units (U/dL) of enzyme activity in the experimental sample were calculated from this standard of Bayer units. After blood collection, the rats were killed by an overdose of anesthesia.

Histology techniques

After blood collection the rats were killed by an overdose of anesthesia and the mandibles were carefully removed and soaked in 10% formalin. Decalcification was carried out in solution of Morse (50 mL of 50% formic acid and 50 mL of 20% sodium citrate). Serial paraffin sections of 5 μm were made on the buccal-lingual aspects of the whole 1st left and right molar and stained with hematoxylin and eosin. Each first lower molar has a mesial-distal diameter of approximately 1 mm, producing approximately 160 sections of 5 μm each.

Histometry of the cementum

Linear measurements were made with the help of a Zeiss microscope at a magnification of 125x using a Sigma computer program (Mocha, Jandel Scientific, San Rafael, CA, USA). From each tooth, 10 measurements were made in sections of 50-µm intervals each. For statistical analysis the mean from each animal was used, calculated from the 20 measurements obtained from the first right and left molars (see Fig.1).

Stereology of bone volume

Volume densities of bone (V_b) and osteoclasts (V_o) were also estimated according to the method described by Shen *et al*. (19). Counting was performed with the aid of a Zeiss microscope at a magnification of 200x. A square lattice of 25 test points was projected into the ocular microscope with the use of a microvid system that connected the microscope to a computer. For each animal, a total of 1200 points were counted distributed equally on apical, buccal and lingual regions (see Fig. 2). A square lattice has 25 points, and 16 sections were necessary to complete the stereometric analysis. The choice for analyzing a total of 1200 points was based on the relative error, described by Weibel (20), established in a pilot experiment of the present study. The distance between the selected sections was 50 µm. V_b and V_o were expressed as percentages of the total points counted.

Statistical analysis

Variance analysis (ANOVA) was used for statistical evaluation. Tukey's test and Student t-test were used to compare differences between groups. $P < 0.05$ was considered significant.

Results

Serum calcium data

Fig. 3 shows the serum calcium levels of the rats treated or not with FK506. After 60 days, the serum calcium levels of the control group and of the experimental group were 9.8±0.1mg/dL and 8.9± 0.2 mg/dL, respectively ($P > 0.05$). After 120 days not observed significant differences when compared with the previous group (9.6±0.3 mg/dL and 8.6±0.9 mg/dL, respectively). After 180 days, the serum calcium levels in the control group were 9.9±0.2 mg/dL whyle that in the group treated with FK506 there was decreased of calcium level (8.9±0.1 mg/dL), however these values were not statistically different ($P > 0.05$). A significant increase in the serum calcium level was observed after 240 days of treatment with FK506 (9.9±0.1mg/dL and 10.7± 0.2 mg/dL; $P < 0.05$). When mean levels were compared in the short periods (60 and 120 days) with those of the long periods (180 and 240 days), a significant increase in serum calcium level was observed in the long periods ($P < 0.05$) (see Fig.4).

Alkaline phosphatase data

Fig. 5 shows the alkaline phosphatase levels of the rats treated or not with FK506. In the control group and in the experimental group, at 60 days, alkaline phosphatase levels were 824±77U/dL and 270.6± 39U/dL, respectively. In the groups at 120 days, alkaline phosphatase levels were 840±47 U/dL for the control group and 254.73±37 U/dL for those treated with FK506. At 180 days, the alkaline phosphatase levels in the control group were 835±63 U/dL and 235±61 U/dL for the FK506 group and at 240 days, the alkaline phosphatase levels were 944±71U/dL and 112.97±37 U/dL for the control group and the experimental group, respectively. These levels were statistically different from those of the controls groups during the same period ($p<0.05$). Fig. 6 showed that the alkaline phosphatase levels decreased significantly ($p<0.05$) in the FK506 groups over long periods.

Histometric findings

Fig. 7 shows the linear measurements (μm ± SD) of the cementum of the first lower molars of normal and treated rats. The cementum of the control rats showed normal morphology at all periods of observation, with linear measurements cervical, medium and apical were: at 60 days (3.9±7; 4.9±5; 10.9±2); 120 days (4.0±3; 5.4±4; 11.2±2); 180 days (4.4±2; 6.2±4; 11.6±6); and 240 days (4.6±3; 6.4±4; 11.9±5), respectively. The linear values of the FK506-treated rats were also similar statistically linear measurements of the control rats at all periods of observation, with measurements cervical, medium and apical were: at 60 days (3.3±2; 5.0±1; 10.8±2); 120 days (4.0±1; 5.5±2; 12.0±3); 180 days (4.6±2; 6.3±1; 11.6±3); and 240 days (5.0±3; 6.6±1; 11.3±2), respectively ($p>0.05$). The cementum of the FK506-treated rats showed normal morphology similar to that of the control rats.

Stereometric findings of alveolar bone

Fig. 8 shows the volumetric densities of alveolar bone (V_b) from the buccal, lingual and apical region of the first mandibular molar in control and FK506 in rats. In control rats, the mean volumetric densities of bone were not statistically different from each other in the different periods of observation, demonstrated: at 60 days (84.1±2; 83.4±3; 66.2±8); 120 days (81.2±3; 84.5±2; 68.3±6); 180 days (89.2±3; 81.3±4; 69.3±4); and 240 days (83.3±4; 84.2±3; 70.1±4), respectively ($p>0.05$). There was not a significant decrease in bone volume at 60 (85.3±1; 80.6±2; 71.3±1), 180 (82.6±2; 84.0±2; 70.0±2) and 240 days (83.6±2; 85.3±2; 71.3±1) of treatment, in comparison with the control group ($p>0.05$). However, at 120 days, the percentage of bone volume in the FK506-treated rats decreased significantly in the lingual and apical region (79.3±3; 62.6±2) and were statistically different from those of the control group ($p<0.05$).

The volumetric densities of osteoclasts (V_o) from the buccal, lingual and apical region of the first mandibular molar in the control and tacrolimus rats are demonstrated in Fig. 9. There was a significant increase in the volumetric densities of the osteoclasts in the FK506-treated rats (4.0±2) when compared with those of the control rats (2.8±4) ($p<0.05$) at 120 days from the apical region, but not at 60 days. At 180 (4.1±1; 4.3±2; 5.3±1) and 240 (4.5±1; 4.3±2; 5.6±1) days from the buccal, lingual and apical region, respectively, the volumetric densities of osteoclasts increased significantly when compared with the short experimental periods (60 (2.3±1; 2.6±2; 2.6±2) and 120 (2.5±1; 4.0±2; 4.0±2) days) ($p<0.05$), but there was no statistically significant different when compared with control group, demonstrated: at 60 days (2.5±3; 3.1±2; 3.3±4); 120 days (2.6±2; 4.2±2; 2.8±4); 180 days (4.3±2; 4.4±4; 5.7±5); and 240 days (4.9±3; 4.0±4; 5.9±4), respectively ($p>0.05$).

Discussion

The present study evaluated the cementum and the alveolar bone alterations following long-term administration of FK506 in a well-characterized animal model. The relevance of this study is the long period of observation of the effects of FK506 on periodontium compared with briefer periods of time (11,13,15).

Experimental protocols in rats are extremely convenient, since more uniform results may be obtained without the interference of other types of treatments. The use of a rat model allows the strict control of some variables, such as gender, age, dose of the drugs, because various side-effects of the FK506 have been difficult to characterize in humans studies, since transplant patients receive combined therapy with FK506 together with corticosteroids or other drugs (21,22).

Not observed any alteration in cementum in the rats molars treated with FK506. The cementum of the FK506-treated rats showed morphology similar was to that of the control rats (Fig. 7). In interesting to point out that in previous

investigations observed that administration of CsA induced new cementum formation and deposition in rat molars(10,23,24). Ayanoglou & Lesty (10) suggested some hypothesis for CsA-induced new cementum, hence the key role in new cementum formation may be played by collagen-producing cells (gingival cementoblasts and fibroblasts) located in the vicinity of the root surface and the mineralization of the abundant collagenous deposits leads to the formation of new cementum. Those hypothesis seem not to apply when use FK506 therapy in rats.

We also evaluated the effect of long-term FK506 therapy on the alveolar bone of rats. Our investigations showed that FK506 administration resulted in striking and unique histomorphometric and serum changes in rat bone mineral metabolism at the earlier experimental periods (120 days). The data presented in this study indicate that FK506 administration for 60 and 120 days leads to an increase in the volumetric densities of osteoclasts (V_o) (Fig. 9) and a decrease in the volumetric densities of alveolar bone (V_b) (Fig. 8). It is believed that in the normal physiological situation, both bone formation and resorption progress in a balanced, regulated manner with osteoclastic bone resorption preceding new bone formation by osteoblasts (15). The mechanisms by which FK506 induces such osteopenia remain unclear, although some hypothesis have been presented. Cunningham (1) postulated that FK506 may exert its osteopenic effect via the T-cell rather than directly on bone.

FK506 may mediate its osteopenic effect by interfering in the cytokine activity on both osteoclasts and osteoblasts at the bone microenvironment (8,13) thus influencing bone remodeling. On the other hand, FK506 might exert a direct trigger effect on bone cells, affecting the osteoblasts more than the osteoclasts (counteracting thus the effects of glucocorticoid), or an indirect effect through cytokines affecting bone cells. In this context, it is of interest, that Inoue *et al*. (11) recently showed that rats given FK506 at a dose used to prevent allograft rejection had no significant reduction in their bone mass.

A time-related improvement occurred at the longer periods of treatment (180 and 240 days) in the volumetric densities of alveolar bone, associated with a significant maintance in the volumetric densities of osteoclasts, demonstrated in Fig. 8 and 9. In contrast, Spolidorio *et al.* (25) showed with CsA occurred increased in the volumetric densities of osteoclasts and decreased in the volumetric densities of alveolar bone in the short periods and maintained in the long periods. The reason for this effect is unknown, although we have hypothesized that the potential advantage of FK506 over CsA on bone mineral density could be explained either by a better induction of IGF-1, a powerful activator of osteoblastic function, a more “bone-friendly” expression of the different T lymphocyte subclasses (26) or by an indirect effect through cytokines affecting bone cells (27).

Bone markers as dynamic parameters of bone metabolism showed a marked increase of bone resorption markers and partially increased bone formation markers indicating a high turnover status. The influence of the FK506 based immunosuppression on bone markers was comparable to a CsA based immunosuppression (28,29).

In the present study, ALP activity was used as an index of osteoblastic activity, and the Ca^{2+} content was used as an index of bone formation. Long-term administration of FK506 caused a high-turnover osteopenia-like state and biochemical date supported this. In initials periods, ALP activity was lower than in the control groups in contrast with Kaihara *et al*. (15), while it was markedly decreased at long-term periods with FK506 in accordance to Goffin *et al.* (30), where demonstrated that FK506 given with low-dose steroids provides a successful immunosuppression with adequate renal function and simultaneously improves bone mass than CsA and normal doses of steroids, but not yet documented in patients given prednisolone. In this context, it is of interest, that results of this study suggest FK506 might exert a direct trigger effect on bone cells, affecting the osteoblasts more than the osteoclasts, or an indirect effect through cytokines affecting bone cells (30).

The Ca^{2+} content at initials periods was not statistically different when compared in the control groups in accordance to Kaihara *et al*. (15), but increased in long-term periods therapy. The reason for this was probably that bone resorption increased more rapidly than bone induction. These findings suggest that FK506 promoted bone formation and resorption also occurred earlier than in the control groups, albeit the action of FK506 binds to FKBP and FK506/FKBP complex inhibits the activation of calcineurin and increases the intercellular Ca^{2+} concentration (15). If FK506 is endowed with such a beneficial effect, the involved mechanism is not yet clear. In experimental animals, FK506, just as CsA, reduces bone mass (13,31), but in contrast with other experimental studies, FK506 may exert favorable effects on bone metabolism, maintance normal bone mineral density (11,30). The data were contradictory with experimental animals with CsA therapy, where ALP activity was increase in all periods, and Ca^{2+} serum maintained similar control groups in all periods (25), demonstrated an imbalance in the metabolism bone with immunosuppressant drugs. In humans, it does not protect bone mass better than CsA after liver or cardiac transplantation, at least in a single investigated lumbar site (7,29).

Within the limits of this experimental study, it may be concluded that FK506 administration has not induced side-effects on the periodontium. Nevertheless, detailed studies are still needed to clarify the possible cellular and molecular mechanisms involved in the diminished effect of immunosuppressive drugs on the periodontium after long-term administration.

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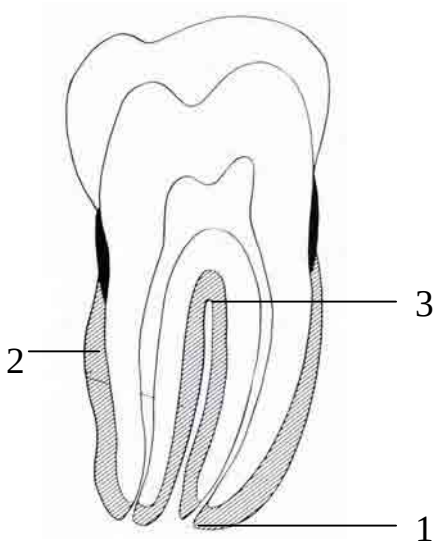


Fig.1 – Schematic illustration showing the regions where linear measurements of the cementum were made.

1- Apical region
2- Medium region
3- Cervical region

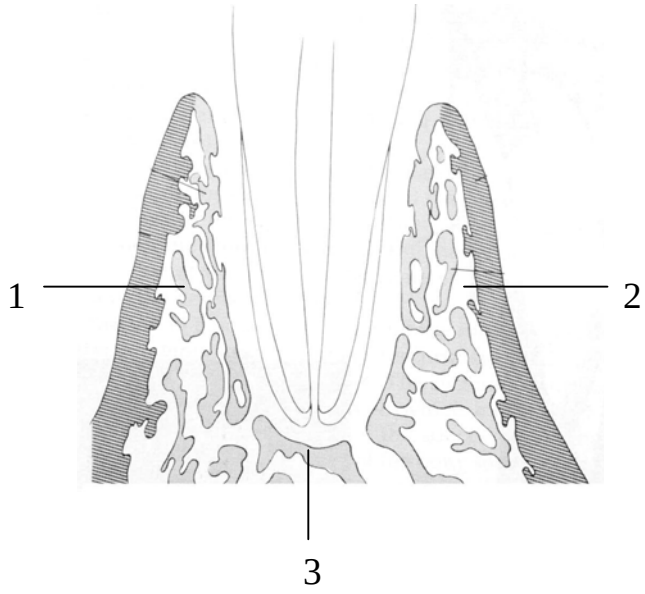
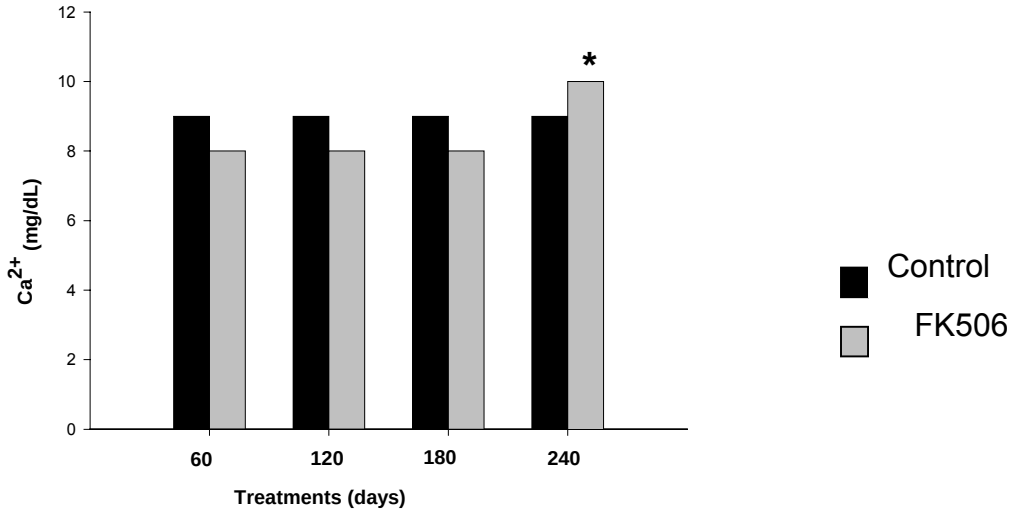


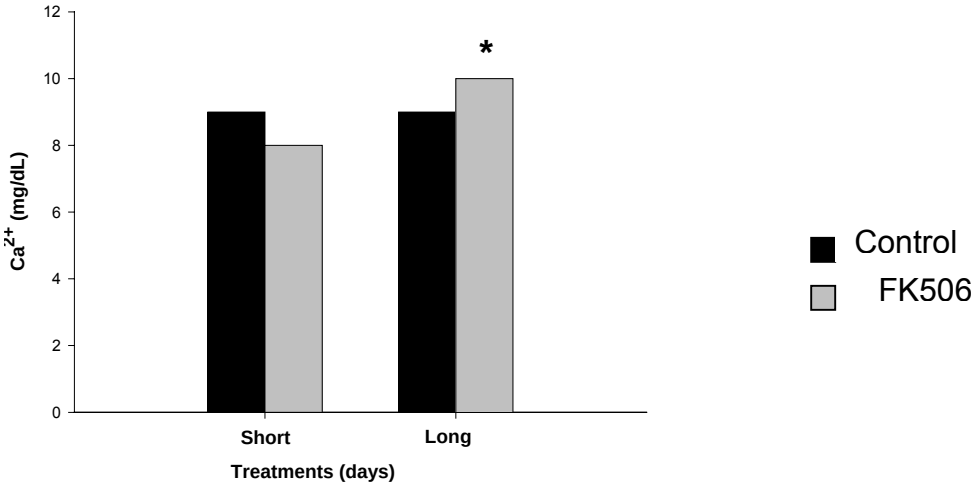
Fig.2- Schematic illustration showing the regions where volumetric densities of the alveolar bone were measured.

1- Buccal bone region
2- Lingual bone region
3- Apical/Interradicular bone region



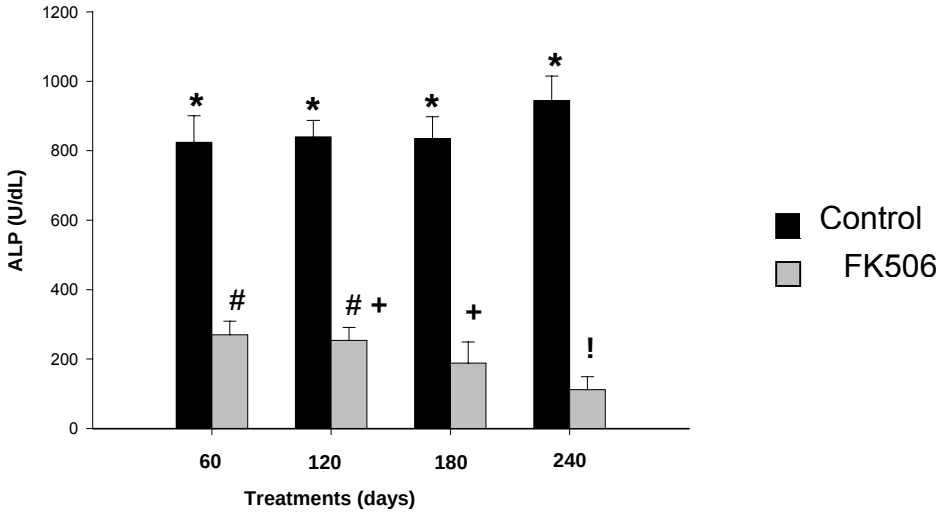
*p<0.05, statistical significance, control group vs. FK506 groups respectively in the same periods.

Fig. 3- Measurements (means ± SD) of the serum calcium levels in the control and FK506 groups during different treatment periods.



*p<0.05, statistical significance, control group vs. FK506 groups respectively in the same periods.

Fig. 4- Measurements (means ± SD) of the serum calcium levels in the control and FK506 groups associating the periods of 60 and 120 days (short periods) and 180 and 240 days (long periods).



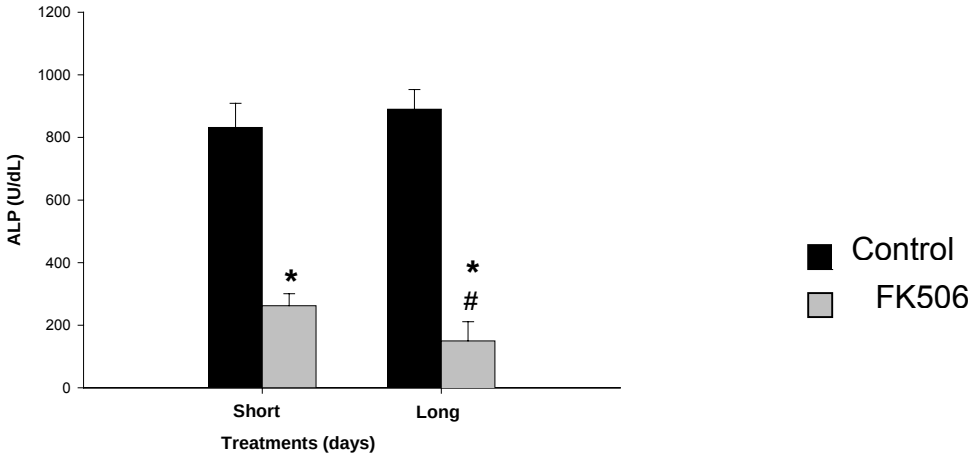
*p<0.05, statistical significance, control group vs. FK506 groups respectively in the different periods.

#p<0.05, statistical significance, FK506groups in the different periods

+p<0.05, statistical significance, FK506groups in the different periods

!p<0.05, statistical significance, FK506groups in the different periods

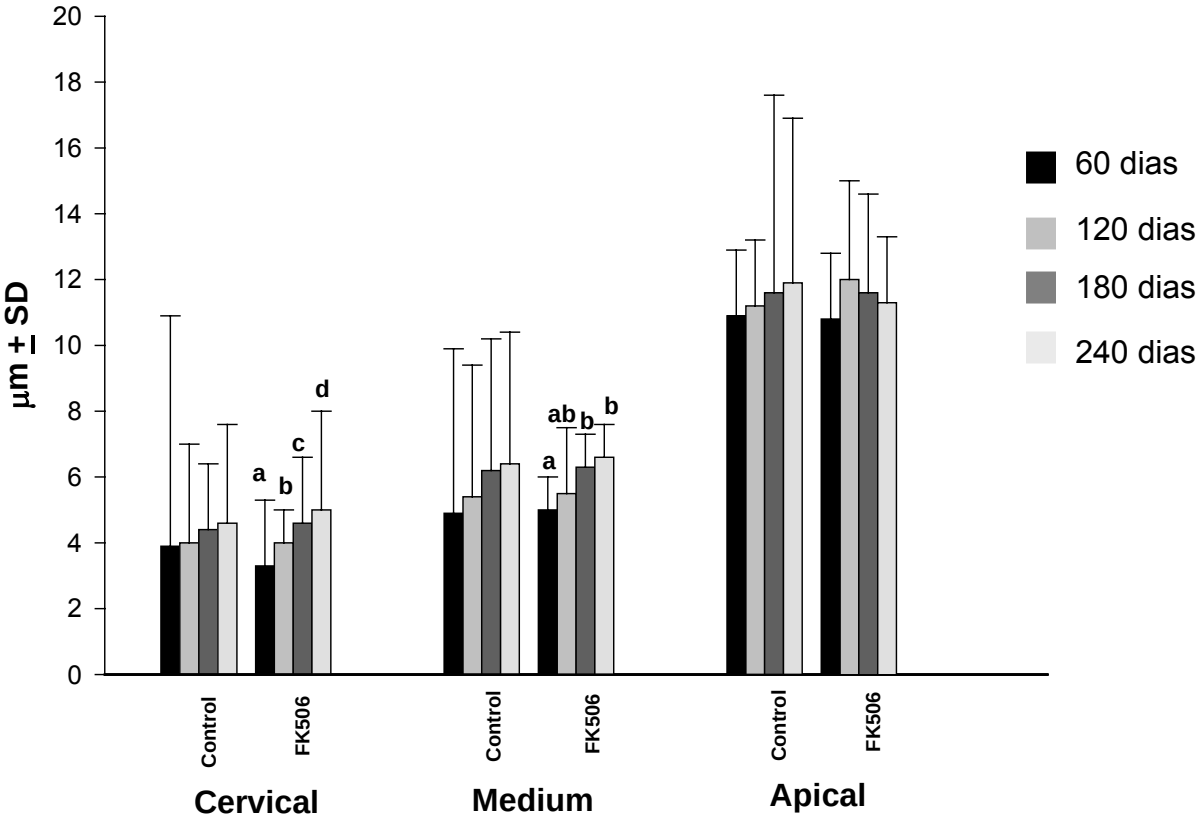
Fig. 5- Measurements (means ± SD) of the serum alkaline phosphatase levels the control and FK506 groups during different treatment periods.



*p<0.05, statistical significance, control group vs. FK506 groups respectively in the same periods.

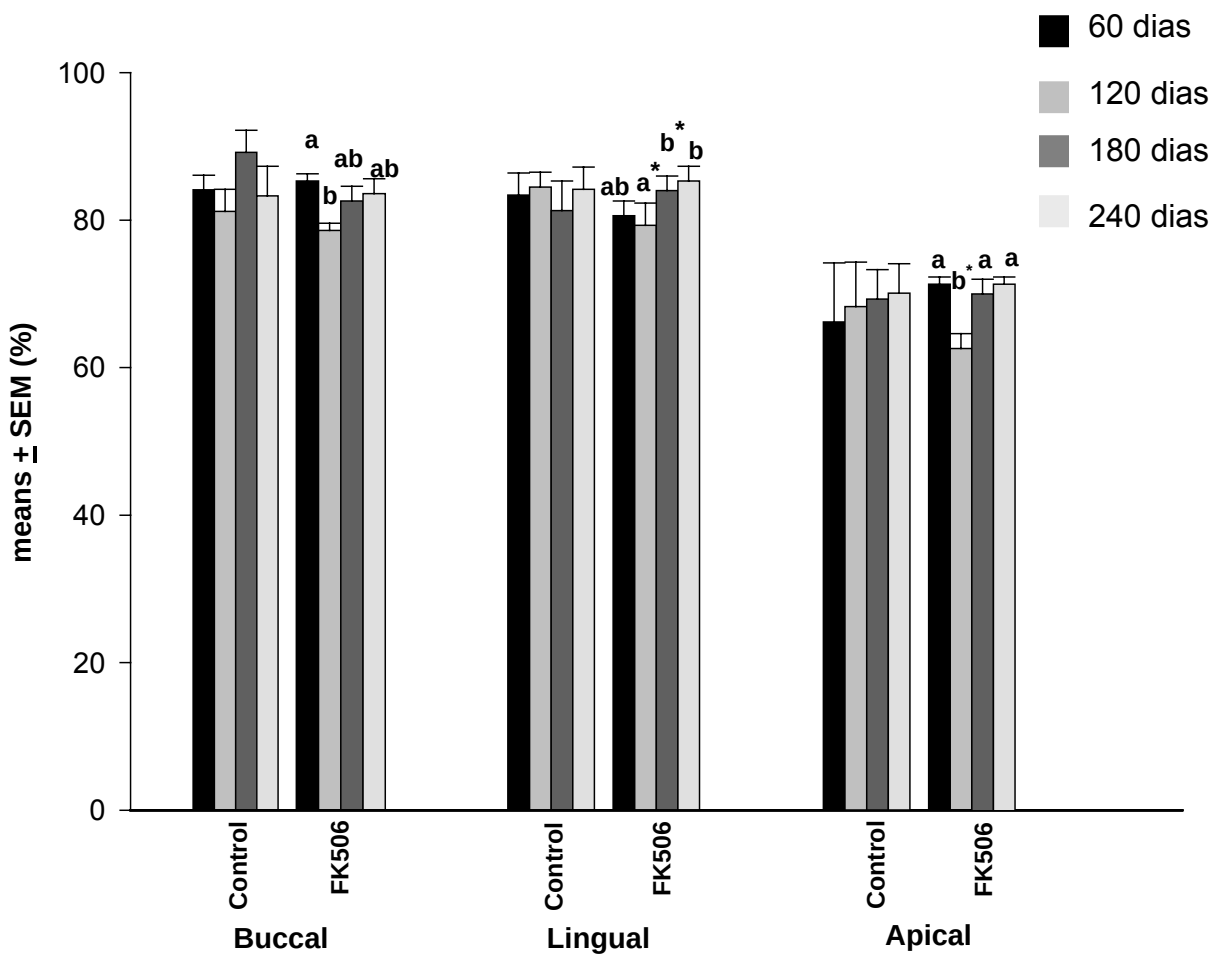
#p<0.05, statistical significance, FK506groups in the different periods

Fig. 6- Measurements (means ± SD) of the serum alkaline phosphatase levels in the control and FK506 groups associating the periods of 60 and 120 days (short periods) and 180 and 240 days (long periods).



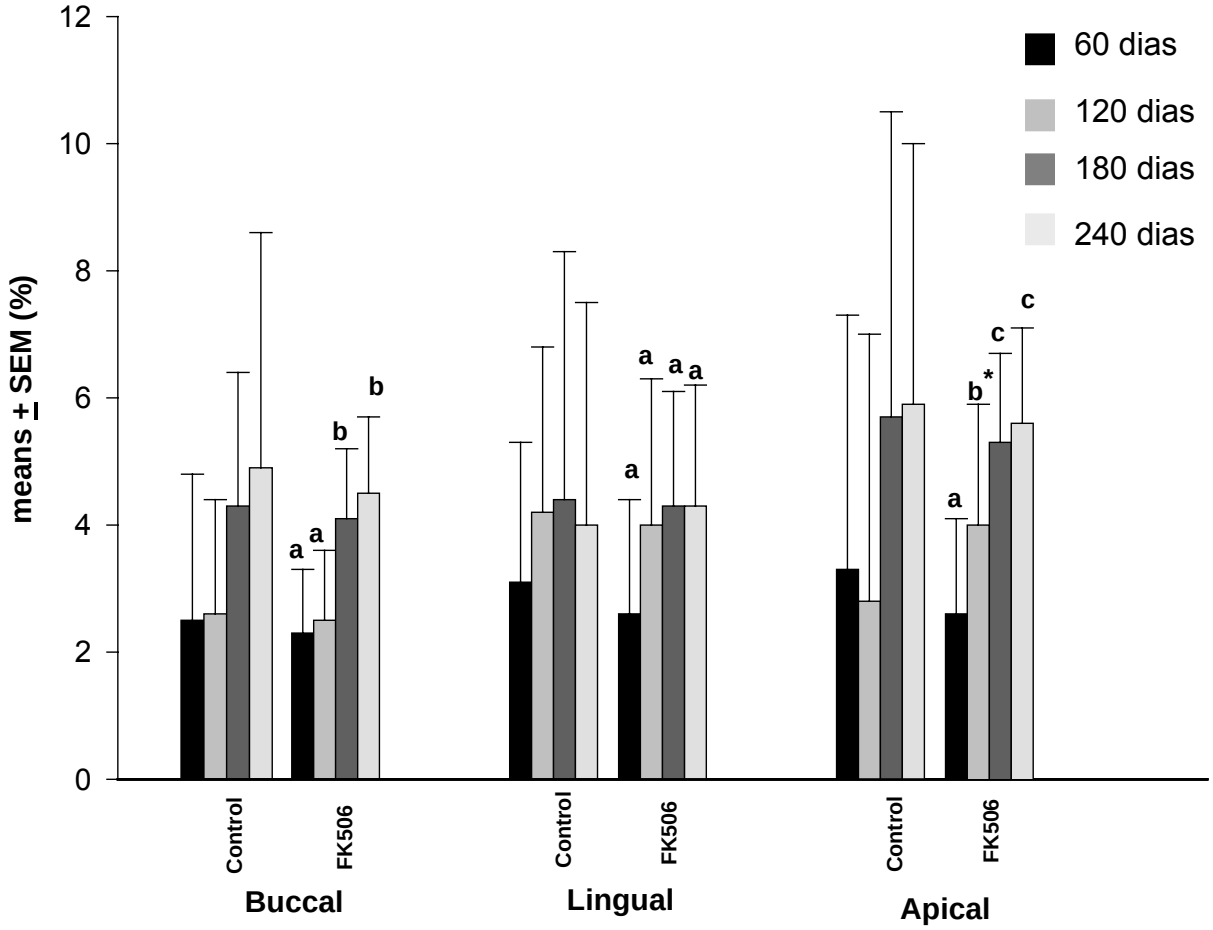
Different letters represent statistically significance difference among means in the same group ($p<0.05$).

Fig. 7- Linear measurements (µm ± SD) of cementum at root surface of first lower molars of normal rats and treated with FK506 in various periods of treatment.



Different letters represent statistically significance difference among means in the same group *p<0.05, statistical significance vs. control rats in the same periods.

Fig. 8- Volumetric densities of alveolar bone from the buccal, lingual and apical region of the first lower molar in control and FK506 rats. Values represent means ± SD. The results are expressed as percentages.



Different letters represent statistically significance difference among means in the same group *p<0.05, statistical significance vs. control rats in the same period.

Fig. 9- Volumetric densities of osteoclasts from the buccal, lingual and apical region of the first lower molar in control and FK506 rats. Values present means ± SD. The results are expressed as percentages.

BIOCHEMICAL EVALUATION IN GLYCEMIC LEVELS OF LONG-TERM FK-506 THERAPY IN RATS.

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Biochemical evaluation in glycemic levels of long-term FK-506 therapy in rats.

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Abstract

One of the more serious complications after transplantation is the development of post-transplantation diabetes mellitus (PTDM), which has a major impact on quality of life, ranging from control of glycemias times to increased susceptibility for infections and cardiovascular complications. It has been suggested that immunosuppressive therapy, mainly FK-506, may be an important factor in the development PTDM. There is a lack of studies exploring the effects of a long-term FK-506 on PTDM in animal's protocols. Therefore, the objective of this study was to evaluate the effects of long-term therapy with FK-506 in rat. Four groups were treated with FK-506 injected subcutaneously in a daily dose of 1mg/Kg body weight. The chosen dose was sufficient to achieve therapeutic FK-506 serum levels. The experimental periods were 60, 120, 180 and 240 days. Four groups were used as controls and received subcutaneous injection of saline solution during all periods. There was a tendency of increase of serum glycemia level in the initial periods (60 and 120 days). However, at 180 and 240 days, the serum glycemia were not statistically different from those of the control group. It can be concluded that the deleterious glycemia effects of FK-506 therapy may be time related side effects.

Introduction

The immunosuppressant drugs (FK-506, mycophenolate mofetil (MMF) and sirolimus) have dramatically improved graft survival transplantation[1]. One of the more serious complications after transplantation is the development of post-transplantation diabetes mellitus (PTDM), which has a major impact on quality of life, ranging from control of glycemias times to increased susceptibility for infections and cardiovascular complications[2]. The influence of diabetes in periodontal issue is being constantly investigated. Although it is difficult to get definitive conclusions on the specific effect of diabetes in periodontal, a variety of alterations is described, including trend to the generalized gingival overgrowth, dental abscesses, periodontitis and dental loss. Perhaps, the major alteration of diabetes not-controlled was reduction of the defense mechanism and increased susceptibility the infections, leading to the destructive periodontal disease[3] or others oral complications. With introduction of the calcineurin inhibitors and the current use of lower doses of steroids the incidence of PTDM has decreased (3-14% of patients). Nevertheless, PTDM remains an important complication after organ transplantation[2]. In that case it seems to be associated to the immunosuppression therapy[1]. It has been suggested that immunosuppressive therapy, mainly FK-506, may be an important factor in the development PTDM. In clinical studies, the effect of FK-506 on PTDM has been studied extensively and revealed contradictory results. However, the independent effects of FK-506 on PTDM have been difficult to characterize in clinical studies, since transplant patients received combined therapy with FK-506 together with corticosteroids[2]. On the other hand, the time of treatment is important. A US Renal Data System (USRDS) evaluation of the incidence, risk factors and outcomes in patients who developed PTDM, estimated the cumulative incidence of PTDM to be 9.1%, 16.0% and 24.0% at 3, 12 and 36 months post-transplant respectively[4]. Contrary to early PTDM, there is limited information associating the prevalence of PTDM with the time of FK-506 therapy[1]. On the other hand, prospective studies after solid organ transplantation with glucose metabolism disorders as the primary endpoint have not been performed until now, although all comparisons between

calcineurin inhibitors concerning PTDM showed a three to five times higher incidence with tacrolimus, many questions remain unanswered because of the different criteria used for the diagnosis of diabetes mellitus (based on insulin requirement) or because of the high trough levels of tacrolimus targeted in the initial studies, albeit data on the mechanism of glucose metabolism disturbance with tacrolimus are more contradictory[1]. Therefore, the objective of this study was to evaluate the effects of long-term therapy with FK-506 in rat.

Material and Methods

Eighty male Holtzman rats (*Rattus novergicus Albinus*) weighing 50g were housed under similar conditions in cages with access to food and water *ad libitum*. The animals were randomly distributed in eight groups of 10 animals each. All protocols described below were approved by the Institutional Experimentation Committee of the School of Dentistry of Araraquara, Araraquara, São Paulo, Brazil. Four groups were treated with FK-506 (Prograf® - Janssen Cilag, Brazil) injected subcutaneously in a daily dose of 1mg/Kg body weight[5-6]. Four groups were used as controls and received subcutaneous injection of saline solution during all periods. The experimental periods were 60, 120, 180 and 240 days. The rats were weighed weekly and monitored for abnormal appearance of coat, abnormal level activity. At the end of experimental periods, the rats were anesthetized with 0,08mg/100g body weight Ketamine (Francotar®, Virbac do Brazil Ind. e Com. Ltda, São Paulo, São Paulo, Brazil) and 4-5ml of blood was obtained by direct cardiac puncture in heparinized capillary tubes for immediate glycemias measurements, using measured colorimetrical (Glicose – PAP Kit, Labtest Ind e Com. Ltda, Ribeirão Preto, São Paulo, Brazil). Levels of FK-506 were determined at the end of each experimental period. After blood collection the rats were killed by an overdose of anesthesia. Variance analysis (ANOVA) was used for statistical evaluation. Tukey's test was used to compare differences between groups. $P < 0.01$ was considered significant.

Results

Fig. 1 show the serum glycemia levels of the control and FK-506 – treated rats. In the control group, the serum glycemia levels ranged between 110.0±1.1mg/dL and 130.0±2.0mg/dL. The FK-506 treated groups showed a discreet increase in the initial period (60 days). However, these values were not statistically different from those of the control group ($p>0.05$). The serum glycemia level of the FK-506-treated rats was significantly increased at 120 days of treatment compared with the control group. At 180 and 240 days the serum glycemia level in the FK-506 – treated rats decreased significantly and was not statistically different from those of the control group.

Serum levels of FK-506 were at the end of experimental periods: 11.4 ±1.3 ng/mL (60 days); 12.6±1.2 ng/mL (120 days); 11.8±1.3 ng/mL (180 days); and 11.2±1.6 ng/mL (240 days). The serum levels of FK-506 was significantly increased at 120 days of treatment compared with the others experimental groups ($p<0.01$) (see Fig. 2).

Discussion

The present study evaluated the diabetes following long-term administration of FK-506 in an animal model. The relevance of this study is the long period of observation of the effects of FK-506 on glycemia levels compared with briefer periods of time. The results of the present work showed that FK-506 administration in an initial period (60 days and 120 days) (Fig.1), in a dose that have been reported to be immunosuppressive (1mg/Kg body weight) [5-6], induced an evident diabetogenic effect, and in addition, the chosen dose 1 mg/Kg body weight was sufficient to achieve therapeutic FK506 serum levels, because this treatment gives estimated peak and trough levels of FK-506 of 11.7±1.3 ng/mL, at means (Fig. 2). This dose is clinically relevant and within the range of doses used in studies on organ and limb transplantation that usually are between 0.6 and 1.0 mg/Kg body weight[7], resulting in consistent responses. Although the exact mechanisms involved in the development of the FK-506-induced diabetes

are not known, there is evidence that this drug inhibits the insulin synthesis via mRNA transcriptional defect or induced hyperinsulinemia promoting insulin resistance[1-8]. Interestingly, in the present study, a gradual time-related improvement was observed at the longer periods of treatment (180 and 240 days). In those periods the values glycemia level were similar to the control rats[9], but we were not the insulin and peptide C levels in this study. These results are in agreement with some prospective longitudinal study in human that showed a relevant role of the time in a reduction of the glycemia level[1]. Those authors suggested that the abnormalities of glucose metabolism may be normalized after prolonged therapy with FK-506. Within the limits of this experimental study, it can be concluded that negative effect of FK-506 therapy on the glycemia could be improved with time. Nevertheless, detailed studies are still needed to clarify the possible cellular and molecular mechanisms involved in the diminished effect of immunosuppressive drugs on the glycemia level after long-term administration.

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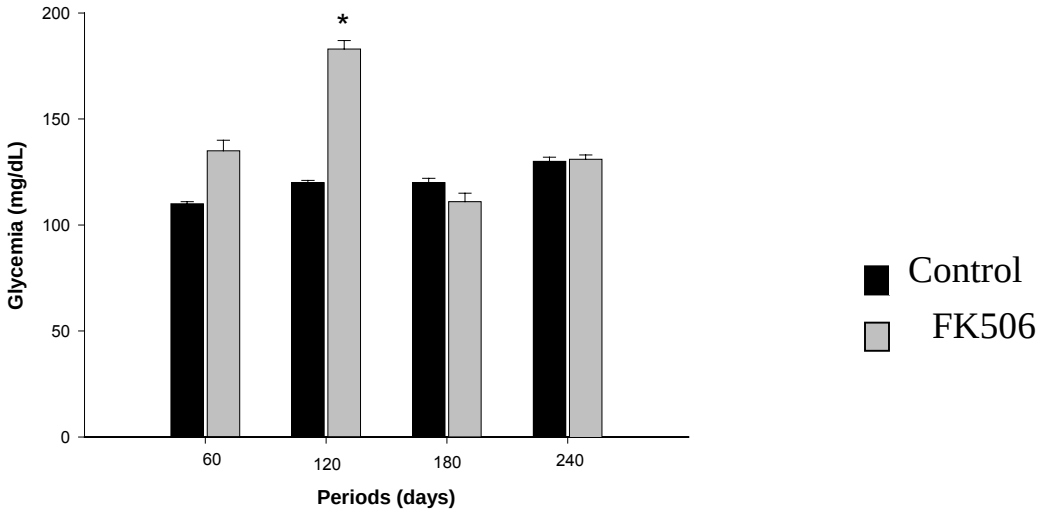
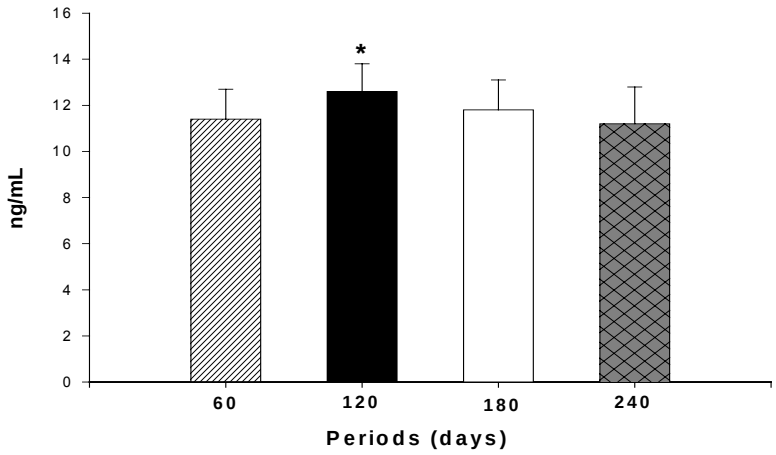


*p<0.01, statistical significance, control group vs. FK-506 groups respectively in the different periods.

Fig. 1- Measurements (means ± standard deviations) of the serum glycemia levels in control group and FK-506 groups in several treatments periods in rats.



*p<0.01, statistical significance, FK-506 groups respectively in the different periods.

Fig. 2- Measurements (means ± standard deviations) of the serum FK506 levels in treated with FK-506 groups in several treatments periods in rats.

DISCUSSÃO

O presente estudo avaliou as alterações nos tecidos periodontais de ratos imunossuprimidos por um longo período de administração de FK506. A relevância do estudo se mostra, principalmente, pelo longo período de observação dos efeitos colaterais do FK506 sobre o periodonto, quando comparado com períodos mais curtos de observação (CVETKOVIC et al., 1994; INOUE et al., 2000; KAIHARA et al., 2002).

O uso de um protocolo experimental em ratos é muito conveniente, uma vez que os resultados obtidos são mais uniformes, pois os mesmos encontram-se livres de outros tipos de tratamento inerentes aos humanos, normalmente associado com corticosteróide ou outras drogas (RODINO e SHANE, 1998; ABDELHADI et al., 2002), sendo ainda estabelecido um bom controle frente a algumas variáveis, como por exemplo, sexo, idade, predisposições genéticas, dose e tempo de tratamento.

À escolha da dose de 1mg/Kg por peso corporal diariamente, foi determinada com base em outros trabalhos da literatura (JIANG et al., 1991, 1995; AKAHANE et al., 1999) e mostrou-se suficiente para as ações terapêuticas do FK506, uma vez que, em média, o nível sérico manteve-se em torno de 11.7±1.3 ng/mL, corroborando com os dados da literatura mais pertinente e recente (VOGGENREITER et al., 2005). Esta dose é clinicamente relevante e está dentro da média da dose usada nos estudos de transplantes de órgãos, que usualmente seria em torno de 0.6 a 1.0 mg/Kg peso corporal, que demonstra apresentar resultados bem consistentes (KAIHARA et al., 2002; LI et al., 2003; FUKUNAGA et al., 2004; MURAMATSU et al., 2005; VOGGENREITER et al., 2005).

Em relação ao tecido gengival de ratos imunossuprimidos por FK506, alguns estudos demonstram que esta mesma droga não induziria ao crescimento gengival (JAMES et al., 2001; McKAIG et al., 2002) ou ainda sugerem que a severidade do crescimento gengival em pacientes imunossuprimidos por FK506 seria menor do que aqueles imunossuprimidos por CsA (BADER et al., 1998; JAMES et al., 2000). Em nosso conhecimento, não há

dados que avaliaram os efeitos sobre o tecido gengival de ratos imunossuprimidos por FK506 por longos períodos.

Em concordância com prévios estudos (HERNANDEZ et al., 2000; JAMES et al., 2000), este trabalho demonstrou que a administração de FK506 por curtos períodos de tratamento (60 e 120 dias) não induziria ao crescimento gengival. Interessante é que neste estudo a administração de FK506 induziu, em todos os ratos nos períodos mais longos (180 e 240 dias), ao crescimento gengival.

A patogênese exata do crescimento gengival induzido por FK506 após longos períodos de tratamento não é conhecida. Nós sugerimos que o crescimento gengival pode ser resultado de uma gradual sensibilização dos fibroblastos gengivais e epitélio gengival. A heterogeneidade dos fibroblastos poderia ser uma das possíveis explicações para a variabilidade de resposta dos tecidos gengivais frente a várias drogas (COTRIM et al., 2003).

Por outro lado, Frizell et al. (1994) verificaram que o FK506 aumenta o colágeno hepático e aumenta os níveis de RNA do fator transformador de crescimento beta 1 e colágenos tipo I, III e IV, reduzindo fibroses hepáticas após 240 dias de tratamento. Algumas citocinas são peptídeos multifuncionais que regulam diversas atividades biológicas incluindo crescimento celular, morte celular ou apoptose, diferenciação celular e sínteses de matriz extracelular (BAUER e SCHUPPAN, 2001). Logo, sugerimos que especial atenção deve ser dada à elucidação dos mecanismos de ação do FK506 sobre a proliferação dos fibroblastos e fibras colágenas, bem como na síntese proteica e atividade colagenolítica em longos períodos de tratamento.

Com relação aos tecidos periodontais de sustentação, a administração de FK506 por longos períodos parece não afetar de forma indesejável estes tecidos. O efeito do FK506 sobre o cemento não demonstrou qualquer alteração, sendo que o cemento de ratos tratados com FK506 apresentou-se com morfologia normal e similar aos ratos dos grupos controles em todos os períodos, diferente da ação de outro imunossupressor, a CsA, que levaria a um

aumento de ilhotas de cemento, com conseqüente aumento da quantidade de cemento (AYANOGLOU, 1998, 1999). Destaca-se que, em nosso conhecimento, este é o primeiro trabalho que avaliou a ação do FK506 sobre o cemento tanto de animais como de humanos.

No presente estudo, nós também avaliamos o quanto os efeitos do FK506 no osso e no seu metabolismo mineral são dependentes do tempo de tratamento, demonstrando que a administração de FK506 resulta em mudanças morfométricas, estereométricas e séricas do metabolismo mineral ósseo de ratos, apenas em períodos experimentais mais curtos (120 dias). Os dados demonstraram que em 60 e 120 dias de administração de FK506, houve um aumento significativo na densidade volumétrica de osteoclastos (V_o) e um decréscimo na densidade volumétrica de osso alveolar (V_b). Em situação fisiológica normal, o progresso de formação e reabsorção óssea está balanceado, mantendo-se regulado com uma reabsorção óssea por osteoclasto precedendo a uma nova formação de osso por osteoblasto (KAIHARA et al., 2002).

Em relação à ação do FK506 sobre o metabolismo ósseo, Cunningham (2005) sugere que o FK506 poderia exercer efeito osteopênico via células-T diretamente sobre o osso. O FK506 poderia mediar este efeito osteopênico por interferência de atividades de citocinas em osteoblastos e osteoclastos (CVETKOVIC et al., 1994; STEMPFLE et al., 2002), influenciando no metabolismo ósseo. Por outro lado, o FK506 deve exercer efeito tóxico direto sobre células ósseas, afetando os osteoblastos mais do que os osteoclastos ou ainda exercendo um efeito indireto através de diminuição na produção de citocinas que afetariam células ósseas (CVETKOVIC et al., 1994; STEMPFLE et al., 2002). Neste contexto, é interessante relatar que Inoue et al. (2000) recentemente demonstraram que FK506 administrado em ratos com dose adequada para prevenir a rejeição de transplantes, não existiu redução significativa de massa óssea, reafirmando a ação conflitante e incerta sobre o metabolismo ósseo.

Em longos períodos (180 e 240 dias) de administração com FK506, ocorreu uma manutenção da densidade volumétrica de osso alveolar, associado com um aumento na densidade volumétrica de osteoclastos, apesar de não ser significativamente diferente quando comparado aos grupos controle dos mesmos períodos. A razão para este efeito é desconhecida, embora nós possamos sugerir que a grande vantagem do FK506 sobre a ação da CsA na densidade mineral óssea, poderia ser explicada pela maior indução de fator crescimento derivado de insulina I (IGF-I), potente ativador de função osteoblástica, provocada pelo FK506 ou por uma maior ação na expressão de diferentes subclasses de linfócitos-T (VOGGENREITER et al., 2000; GOFFIN et al., 2003).

Marcadores ósseos são usados como parâmetros dinâmicos do metabolismo ósseo, demonstrando o equilíbrio entre reabsorção e formação óssea indicando o estado de remodelação óssea. A influência de um regime baseado na imunossupressão por FK506, poderia ser comparada a influência de um regime de imunossupressão baseado em CsA através da ação nesse metabolismo ósseo (SHANE et al., 1997; STEMPFLE et al., 1999).

No presente estudo, a atividade de fosfatase alcalina foi usada como um índice de atividade osteoclástica e o nível sérico de cálcio foram usados como índice de formação de osso. A administração por longos períodos de FK506 demonstrou um alto desequilíbrio do metabolismo ósseo, pois desde os períodos iniciais (60 e 120 dias) houve diminuições nos níveis de fosfatase alcalina em comparação aos grupos controle de mesmo período, contrastando com Kaihara et al. (2002), enquanto que em maiores períodos (180 e 240 dias) ocorreu uma marcada diminuição mesmo comparada com períodos iniciais, sendo tudo em concordância com Goffin et al. (2002), onde demonstraram que o FK506 em doses adequadas causaria um sucesso na imunossupressão com adequada função renal e simultaneamente manutenção de massa óssea, sugerindo que o FK506 poderia afetar mais a atividade osteoblástica do que a osteoclástica (GOFFIN et al., 2002).

Em relação aos níveis séricos de cálcio, nos períodos iniciais não houve alteração significativa em relação aos grupos controles em concordância com Kaihara et al. (2002), mas aumentaram significativamente nos períodos longos. A razão poderia ser explicada provavelmente por um aumento no metabolismo ósseo, sugerindo que o FK506 promoveria uma maior formação óssea em relação à reabsorção corroborando com outros estudos que demonstraram o FK506 exerce efeitos favoráveis no metabolismo, mantendo a densidade óssea mineral normal (INOUE et al., 2000; GOFFIN et al., 2002).

Em relação aos níveis séricos de glicose, o nosso estudo avaliou o efeito da administração em longo período de FK506 em animais modelos, sendo a sua relevância a comparação com períodos mais curtos de tratamento. Os resultados demonstraram que nos períodos mais curtos (60 e 120 dias) de administração com FK506, houve um evidente efeito diabetogênico. Embora o exato mecanismo envolvendo este desenvolvimento não ser conhecido, há evidências que o FK506 poderia inibir a síntese de insulina via RNA mensageiro ou induzir a uma hiperinsulinemia promovendo resistência à insulina (MAES et al., 2001; VAN HOFF et al., 1999). Nos períodos mais longos (180 e 240 dias) de administração de FK506, os níveis séricos de glicose foram similares aos grupos controles de mesmo período (MITRUKA e RAWNSLEY, 1977), sendo que estes resultados estão de acordo com estudos prospectivos em humanos que demonstraram que o tempo de administração possui um papel relevante na redução dos níveis de glicemia (MAES et al., 2001), sugerindo que anormalidades nos níveis de glicose podem ser normalizadas após terapia prolongada com FK506.

CONCLUSÕES

Dentro dos limites destes estudos, e através destes resultados podemos concluir que:

- O tempo de tratamento com FK506 é um fator importante no desenvolvimento dos aumentos gengivais
- O aumento gengival induzido pelo FK506 está associado com aumento de fibroblastos e matriz extracelular.
- A terapia com FK506 pode induzir alterações nas concentrações séricas de cálcio e de fosfatase alcalina, nos mais longos períodos de tratamento, sugerindo um desequilíbrio no metabolismo ósseo.
- A terapia com FK506, em longos períodos, não induziu alterações estereométricas e morfométricas em cemento e osso alveolar.
- O efeito negativo da terapia com FK506 nos níveis de glicemia, poderia ser melhorado com o tempo.

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RESUMO

NASSAR, C.A. Avaliação morfométrica e estereométrica dos tecidos periodontais de ratos imunossuprimidos por Tacrolimus (FK506). 2006. 126f. Tese (Doutorado em Periodontia) – Faculdade de Odontologia, Universidade Estadual Paulista, Araraquara, 2006.

Tacrolimus (FK506) é uma droga imunossupressora usada em transplantes de órgãos e também como terapia alternativa em pacientes com rejeição refratária de enxertos ou intolerância à ciclosporina A (CsA), sem os efeitos adversos atribuídos frequentemente à CsA. Atualmente não há estudos que explorem os efeitos da administração de FK506 por longos períodos sobre o periodonto. Ë sugerido também que terapias imunossupressivas, principalmente com FK506, possam ser um importante fator no desenvolvimento de diabetes mellitus pós-transplantes (PTDM). Existem alguns estudos explorando os efeitos do FK506 por longos períodos no desenvolvimento da PTDM em protocolos animais. Assim os objetivos do estudo foram avaliar os efeitos da terapia com FK506 por longos períodos sobre os tecidos gengivais, metabolismo do osso alveolar e do cemento de ratos, bem como os efeitos sobre os níveis glicêmicos. Quatro grupos, com 10 ratos cada, foram tratados com FK506 injetados subcutaneamente em doses diária de 1mg/Kg por peso corporal. A escolha dessa dosagem foi suficiente para que o FK506 atingisse os níveis séricos terapêuticos. Os períodos experimentais foram de 60, 120, 180 e 240 dias. Quatro grupos, com 10 ratos cada, foram usados como controles nos mesmos períodos experimentais e recebeu subcutaneamente, injeção de solução salina durante todos os períodos. Ao final de cada período experimental, coletas de sangue foram realizadas para medir os níveis séricos de glicemia, cálcio e fosfatase alcalina (ALP). Após o processamento histológico, o tecido conjuntivo e epitélio oral, bem como a densidade volumétrica de fibroblastos (Vf), fibras colágenas (Vcf) e outras estruturas (Vo) e ainda osso alveolar e cemento, bem como densidade volumétrica de osso (V_b) e osteoclastos (V_o) foram medidas na região primeiro molar inferior. Após 180 e 240 dias de tratamento com FK506, houve evidente crescimento gengival associado com um significante aumento de

tecido epitelial e conjuntivo. Houve ainda um aumento de Vf, Vcf e Vo. Com relação ao metabolismo ósseo, houve uma tendência de diminuição, estatisticamente significante, dos níveis de ALP em todos os períodos tratados com FK506, sendo que com os níveis de cálcio apenas um aumento em 240 dias de tratamento, quando comparados com os grupos controles. Após 60, 180 e 240 dias de tratamento com FK506 não houve alterações em V_b e V_o. Após 120 dias de tratamento com FK506 houve sim evidente aumento de V_b associado a um aumento de V_o, mas não observado perda de osso alveolar. Houve ainda uma tendência de aumento nos níveis séricos de glicemia em curtos períodos (60 e 120 dias), com posterior tendência a normalidade nos períodos mais longos de tratamento com FK506. Assim, nós podemos concluir que o FK506 em longos períodos pode levar os efeitos deletérios sobre os tecidos gengivais e pode ser dependente da idade, mas não causaria efeitos negativos sobre o osso alveolar e nem ao cemento. E ainda em relação aos níveis séricos de glicemia, os efeitos negativos da terapia com FK506 também são dependentes do tempo.

Palavras-chave: Tacrolimo; doenças periodontais; gengiva; cemento dentário; osso alveolar

ABSTRACT

NASSAR, C.A. Evaluation of morphometric and stereometric of periodontal tissues at immunosuppressed of Tacrolimus (FK506). 2006. 126f. Tese (Doutorado em Periodontia) – Faculdade de Odontologia, Universidade Estadual Paulista, Araraquara, 2006.

Tacrolimus (FK506) is an immunosuppressive drug in organ transplantation and it is also an alternative therapy used for transplant patients with either refractory graft rejection or intolerant to cyclosporine A (CsA), without the adverse effects frequently attributed to CsA therapy. Nevertheless there are not studies exploring the effects of long-term FK506 therapy on periodontium. It has been suggested that immunosuppressive therapy, mainly FK506, may be an important factor in the development of post-transplantation diabetes mellitus (PTDM). There is a lack of studies exploring the effects of a long-term FK506 on PTDM in animal's protocols. The objectives of the present study were to evaluate the effects of long-term therapy with FK506 on the gingival tissue, alveolar bone metabolism and cementum in rats and the deleterious glycemic effects of long-term therapy with FK506. Four groups, 10 rats each, were treated with FK506 injected subcutaneously in a daily dose of 1mg/Kg body weight. The chosen dose was sufficient to achieve therapeutic FK506 serum levels. The experimental periods were 60, 120, 180 and 240 days. Four groups, 10 rats each, were used as controls and received subcutaneous injection of saline solution during all periods. At the end of period experimental, blood collection was obtained and serum levels glycemic, calcium and alkaline phosphatase (ALP) were measured. After histological processing, the oral and connective tissue, as well as, volume densities of fibroblasts (Vf), collagen fibers (Vcf) and others structures (Vo) and the alveolar bone and cementum, as well as, volume densities of bone (V_b) and osteoclasts (V_o) were assessed at the region of the lower first molar. After 180 and 240 days of treatment with FK506, evident gingival overgrowth associated a significant increase of epithelium and connective tissue was observed. There was an increase of Vf, Vcf and Vo. There was a tendency of decrease of ALP level in all periods with FK506, statistically significant, but calcium serum level

showed increase so long period (240 days) when compared with control group. After 60, 180 and 240 days of treatment with FK506 not observed V_b and V_o alterations. After 120 days of treatment with FK506, evident increase V_b associated a significant increase V_o was observed, but it was not showed alveolar bone loss. There was a tendency of increase of serum glycemia level in the initial periods (60 and 120 days). However, at 180 and 240 days, the serum glycemia were not statistically different from those of the control group. Within the limits of this experimental study, it can be concluded that the deleterious gingival effects of FK506 administration may be time and age related side effects and that have not the negative effects of FK506 administration long-term on the alveolar bone and cementum. It can be concluded that the deleterious glycemia effects of FK506 therapy may be time related side effects.

Keywords: Tacrolimus; periodontal diseases; gingiva; dental cementum; alveolar bone.