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NICOLLY PARENTE RIBEIRO FROTA

EFEITOS DA ADMINISTRAÇÃO DO BISFOSFONATO TILUDRONATO, ASSOCIADO OU NÃO À TERAPIA PERIODONTAL MECÂNICA, NA PERIODONTITE EXPERIMENTAL EM RATOS

FORTALEZA

2013

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Dissertação apresentada à coordenação do Programa de Pós-Graduação em Odontologia como requisito parcial para obtenção do título de Mestre em Odontologia

Área de concentração: Clínica Odontológica

Orientadora: Profa. Dra. Flávia Aparecida Chaves Furlaneto

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Data de aprovação:__/__/__

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Para ser grande, sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa. Põe quanto és
No mínimo que fazes.
Assim em cada lago a lua toda
Brilha, porque alta vive

Fernando Pessoa

RESUMO

Introdução e Objetivos: A eficácia comprovada dos bisfosfonatos em inibir a reabsorção óssea osteoclástica levou à utilização dos mesmos no tratamento da periodontite. Esta dissertação, composta por 2 artigos, teve como objetivos: (1) avaliar, histologicamente, os efeitos da administração sistêmica do bisfosfonato Tiludronato (TIL) na periodontite induzida por ligadura em ratos; (2) avaliar, histologicamente, os efeitos da administração sistêmica do TIL como terapia adjuvante ao tratamento periodontal mecânico na periodontite induzida por ligadura em ratos. **Métodos:** No estudo 1, 32 ratos adultos machos foram divididos em 4 grupos (n=8): C, DP, DP-TIL5 e DP-TIL15 (C-grupo Controle, DP-grupos Periodontite). Nos grupos DP, ligaduras foram colocadas na área cervical dos 1os molares inferiores direitos de cada um dos ratos no 1º dia. Após 15 dias, soluções de TIL (Tildren®, Ceva Saúde Animal Ltda., Paulínia/SP, Brasil) nas dosagens de 5 mg/kg de peso corporal (grupo DP-TIL5) e 15 mg/kg de peso corporal (grupo DP-TIL15) foram administradas, 5 vezes por semana, durante 3 semanas. No estudo 2, 40 ratos adultos machos foram divididos em 5 grupos (n=8): C, DP, DPT, DPT-TIL5 e DPT-TIL15. Nos grupos DP, foram colocadas ligaduras, conforme descrição anterior. Após 15 dias, as ligaduras dos ratos dos grupos DPT, DPT-TIL5 e DPT-TIL15 foram removidas, e foram realizados raspagem e alisamento radicular. Soluções de TIL nas dosagens de 5 mg/kg de peso corporal (DPT-TIL5) e 15 mg/kg de peso corporal (DPT-TIL15) foram administradas, 5 vezes por semana, durante 3 semanas. Os animais foram submetidos à eutanásia no 36º dia. Foram realizadas análises histológica qualitativa e histométrica. Os dados obtidos foram analisados estatisticamente (ANOVA, Tukey, $p< 0,05$). **Resultados**: No estudo 1, a perda óssea alveolar foi significativamente reduzida no grupo DP-TIL5 (1,12 mm±0,24), quando comparada à dos grupos DP (1,70 mm±0,32) e DP-TIL15 (1,47 mm±0,21). Os animais dos grupos DP apresentaram maior perda de inserção quando comparados aos do grupo C (0,12 mm±0,09). Não houve diferenças na perda de inserção entre os grupos DP (DP: 0,53 mm±0,19; DP-TIL5: 0,37 mm±0,09; DP-TIL15: 0,52 mm±0,13). No estudo 2, não houve diferenças na perda óssea alveolar entre os grupos DPT (1,27 mm±0,15), DPT-TIL 5 (1,18 mm±0,10) e DPT-TIL 15 (1,26 mm±0,40). A perda óssea alveolar observada nesses grupos foi menor que a do grupo DP e não diferiu estatisticamente da perda óssea alveolar encontrada no grupo C. Todos os animais dos grupos com ligadura (grupo DP: 0,59 mm±0,16; grupo DPT: 0,39 mm±0,07; grupo DPT-TIL 5: 0,42 mm±0,05; grupo DPT-TIL 15: 0,48 mm ± 0,09) apresentaram perdas de inserção estatisticamente maiores que os animais do grupo C (0,12 mm±0,09). Os grupos DPT e DPT-TIL 5

apresentaram menor perda de inserção que o grupo DP. **Conclusões:** Dentro dos limites deste estudo, pode ser concluído que (i) a administração sistêmica de TIL reduziu a perda óssea alveolar na periodontite estabelecida em ratos; (ii) a dosagem do TIL pode influenciar suas propriedades antirreabsortivas e anti-inflamatórias; (iii) a administração sistêmica de TIL não proporcionou benefícios adicionais à terapia periodontal mecânica em ratos com periodontite experimental.

Palavras-chave: Difosfonatos. Periodontite. Osso e Ossos. Reabsorção Óssea. Raspagem Dentária.

ABSTRACT

Background and Objectives: The proven efficacy of bisphosphonates to inhibit the osteoclastic bone resorption has led to their use in the management of periodontal diseases. This dissertation, comprised by 2 manuscripts, aimed: (1) to histologically analyze the effects of systemic administration of Tiludronate (TIL) on ligature-induced periodontitis in rats; (2) to histologically analyze the effects of systemic administration of TIL as an adjunctive therapy to mechanical periodontal treatment on ligature-induced periodontitis in rats. **Methods:** In study 1, 32 adult male rats were divided into four groups (n=8): C, PD, PD-TIL5, PD-TIL15 (C–Control group, PD–Periodontitis groups). On PD groups, a ligature was placed in the cervical area of the right mandibular 1st molar of each rat. After 15 days, TIL solutions (Tildren®, Ceva Saúde Animal Ltda., Paulínia, SP, Brazil) at dosages of 5 mg/kg body weight (group PD-TIL5) or 15 mg/kg body weight (group PD-TIL15) were subcutaneously administered 5 times a week for 3 weeks. In study 2, 40 adult male rats were divided into five groups (n=8): C, PD, PDT, PDT-TIL 5, PDT-TIL 15. On PD groups, ligatures were placed as described. After 15 days, ligatures of the rats from groups PDT, PDT-TIL5 and PDT-TIL15 were removed and scaling and root planing were performed. TIL solutions at dosages of 5 mg/kg body weight (group PDT-TIL5) or 15 mg/kg body weight (group PDT-TIL15) were subcutaneously administered 5 times a week for 3 weeks. All animals were euthanized at the 36th day. Histometric and histologic analyses were performed. Data were statistically analyzed (ANOVA, Tukey, $p<0.05$). **Results:** In study 1, alveolar bone loss was significantly reduced in group PD-TIL5 (1.12 mm±0.24), when compared with groups PD (1.70 mm±0.32) and PD-TIL15 (1.47 mm±0.21). The animals from all PD groups presented more periodontal attachment loss than the ones from group C (0.12 mm±0.09). There were no differences in periodontal attachment loss among PD groups (PD: 0.53 mm±0.19; PD-TIL5: 0.37 mm±0.09; PD-TIL15: 0.52 mm±0.13). In study 2, there were no differences in alveolar bone losses among groups PDT (1.27 mm±0.15), PDT-TIL 5 (1.18 mm±0.10) and PDT-TIL 15 (1.26 mm±0.40). The alveolar bone losses found in these groups were slighter than the alveolar bone loss observed in group PD and did not statistically differ from the alveolar bone loss found in group C. Animals from all groups with periodontitis induction (group PD: 0.59 mm±0.16; group PDT: 0.39 mm±0.07; group PDT-TIL 5: 0.42 mm±0.05; group PDT-TIL 15: 0.48 mm ± 0.09) presented periodontal attachment losses statistically greater than the animals from group C (0.12 mm±0.09). Groups PDT and PDT-TIL 5 presented less periodontal attachment loss than group PD. **Conclusions:** Within the

limits of this study, it can be concluded that (i) systemically-administered TIL solution reduced alveolar bone loss in established periodontitis in rats, (ii) dosage of TIL may influence its anti-inflammatory and anti-resorptive properties and (iii) systemically-administered TIL did not result in additional benefits to periodontal mechanical therapy in rats with experimental periodontitis.

Keywords: Diphosphonates. Periodontitis. Bone and Bones. Bone Resorption. Dental Scaling.

SUMÁRIO

1 INTRODUÇÃO GERAL

O conhecimento sobre a patogênese das doenças periodontais evoluiu consideravelmente nos últimos 45 anos, desde que foi relatado, pela primeira vez, que o biofilme bacteriano exerce uma função importante no estabelecimento e na progressão das doenças periodontais[1]. A presença de um biofilme dental periodontopatogênico pode induzir uma reação inflamatória nos tecidos periodontais, com a secreção de citocinas pró-inflamatórias, como Interleucina-1β (IL-1β) e Fator de Necrose Tumoral-α (TNF-α), de prostaglandinas e Metaloproteinases de Matriz (MMPs), por células imunes (leucócitos e macrófagos) e também por fibroblastos gengivais[2; 3]. Esses mediadores inflamatórios estimulam a reabsorção óssea alveolar realizada por osteoclastos e também a migração apical do epitélio juncional, caracterizando a periodontite[4].

Os pré-requisitos para um tratamento periodontal convencional bem-sucedido são a cooperação do paciente, uma adequada higiene oral[5] e o debridamento mecânico das superfícies dentárias[6; 7]. Em indivíduos geneticamente suscetíveis e/ou na presença de fatores de risco imuno-reguladores, como o diabetes melito e o uso de tabaco, a severidade e a progressão da doença periodontal são modificadas[8], o que pode dificultar o controle da doença. A importância reconhecida da resposta inflamatória do hospedeiro na patogênese da periodontite representa uma oportunidade de explorar novas estratégias de tratamento[9].

Três categorias principais de modulação da resposta do hospedeiro vêm sendo estudadas na terapia periodontal: antiproteinases (representadas pelas tetraciclinas), medicamentos anti-inflamatórios e fármacos que inibem a reabsorção óssea, representados por agentes antirreabsortivos, como os bisfosfonatos (BFs)[10; 11].

Os BFs são fármacos sintéticos químicos muito eficazes no tratamento de algumas patologias ósseas, como osteoporose, doença de Paget, mieloma múltiplo, hipercalcemia de malignidade e metástases ósseas, diminuindo o risco de fraturas[12; 13]. Existem três gerações de BFs conhecidas, sendo que a potência dos fármacos aumenta da primeira à terceira geração[10]. A primeira geração possui cadeias laterais alquila (por exemplo, o etidronato), a segunda geração possui cadeia lateral amino-terminal (como exemplo, o alendronato), e a terceira geração possui uma cadeia lateral cíclica (por exemplo, o zoledronato)[4]. Parecem existir diferenças quanto à ação dos BFs na periodontite dependendo do tipo de bisfosfonato (BF) utilizado, de acordo com suas cadeias laterais[14], e também dependendo da dosagem do BF[15] e do tempo de duração da terapia[16].

A eficácia comprovada dos BFs em inibir a reabsorção óssea osteoclástica[17] levou à utilização dos mesmos no tratamento da periodontite, agindo como um fator modulador da resposta do hospedeiro[18; 19]. Alguns BFs também apresentam atividade antibacteriana[20; 21] e efeito anti-inflamatório[20; 22; 23; 24]. Em virtude dessas ações, os BFs parecem ser uma alternativa promissora no tratamento periodontal e têm sido avaliados em estudos em animais e em seres humanos[4; 11; 19].Os BFs foram avaliados quando administrados localmente[15; 19; 25; 26; 27; 28]e sistemicamente[11; 16; 20; 24; 29; 30; 31]em vários estudos. Embora seja difícil comparar dados de diferentes publicações porque diversos tipos e doses de BFs foram utilizados, os estudos mostraram um benefício óbvio dos BFs na doença periodontal, resultando em reabsorção óssea alveolar reduzida[29; 30; 32] juntamente com benefícios clínicos e/ou histológicos na resolução da inflamação em tecidos periodontais[20; 22; 23; 33; 34; 35].

Especificamente em relação aos estudos clínicos em humanos, benefícios adicionais foram demonstrados quando os BFs foram associados ao debridamento mecânico, em comparação com o debridamento mecânico isoladamente[18; 25; 28; 34; 35; 36; 37]. Esses benefícios caracterizam-se principalmente pela redução da perda óssea alveolar, pelo aumento da densidade mineral óssea e pela redução de profundidades de sondagem[18; 19; 26; 28; 34; 35; 37].

O Tiludronato (4-clorofenil tiometileno-1,1-bisfosfonato), um BF de 1ª geração, foi caracterizado por exercer atividade inibitória dose-dependente na reabsorção óssea em diversos estudos pré-clínicos *in vivo,* incluindo modelos de ratos *tiroparatireoidectomizados*[38], neurectomizados[39]e ratas ovariectomizadas[38]. Estudos *in vitro* demonstraram que esse BF também possui ação anti-inflamatória, podendo inibir a liberação de IL-6 por osteoblastos[40] e a secreção de IL-1ß, IL-6, óxido nítrico (NO) e TNF-α por macrófagos ativados, de maneira dose-dependente[41]. Também foi demonstrada a ação inibitória do Tiludronato (TIL) sobre enzimas importantes no processo de degradação de componentes da matriz extracelular na periodontite (MMP-1 e MMP-3) em cultura de células de ligamento periodontal humano[42]. Por não conter nitrogênio em sua formulação, o TIL não apresenta os efeitos adversos comumente associados ao uso de BFs nitrogenados, como lesões oculares[43], irritação gastrointestinal[44], desenvolvimento da resposta de fase aguda[44] e osteonecrose dos maxilares[45; 46; 47; 48; 49]. O TIL é um composto seguro, com margens terapêuticas apreciáveis[50; 51]. Devido às características antirreabsortivas, anti-inflamatórias e de segurança do TIL, é possível que ele seja um BF eficiente para uso na periodontite.

Recentemente, demonstrou-se que a aplicação local de TIL na gengiva palatal de ratos com periodontite levou à diminuição da perda óssea alveolar[52]. Pelo estado atual do

conhecimento, não há estudos avaliando os efeitos da administração sistêmica do TIL, associado ou não ao tratamento periodontal mecânico, na periodontite.

2 PROPOSIÇÃO

Os objetivos deste estudo foram:

1- Avaliar, histomorfometricamente, os efeitos da administração sistêmica do Tiludronato na periodontite induzida por ligadura em ratos;
2- Avaliar, histomorfometricamente, os efeitos da administração sistêmica do Tiludronato, como terapia adjuvante ao tratamento periodontal mecânico, na periodontite induzida por ligadura em ratos.

3 CAPÍTULOS

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado. Os capítulos contêm cópias de artigos científicos de autoria do candidato, redigidos de acordo com as normas das revistas científicas escolhidas para as devidas publicações.

Por se tratar de pesquisa envolvendo animais, o projeto de pesquisa referente a esta dissertação foi submetido à apreciação da Comissão de Ética em Pesquisa Animal (CEPA) da Universidade Federal do Ceará, tendo sido aprovado (Anexo 1). Assim sendo, esta dissertação é composta por dois capítulos, contendo artigos a serem submetidos para publicação em revistas científicas, conforme descrito abaixo:

- **Capítulo 1:**

"Administration of Tiludronate Reduces Alveolar Bone Loss in Rats with Ligature-Induced Periodontitis." Frota NPR, Furlaneto FAC.

Este artigo será submetido para publicação no periódico "Journal of Periodontology".

- **Capítulo 2:**

"Effects of Tiludronate Administration as an Adjunctive to Mechanical Periodontal Treatment in Experimental Periodontitis in Rats." Frota NPR, Furlaneto FAC.

Este artigo será submetido para publicação no periódico "Journal of Periodontology".

CAPÍTULO 1:

"Administration of Tiludronate Reduces Alveolar Bone Loss in Rats with Ligature-Induced Periodontitis."

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ABSTRACT

Background: The proven efficacy of bisphosphonates to inhibit the osteoclastic bone resorption has led to their use in the management of periodontal diseases as a host modulating factor. The purpose of this study was to histologically analyze the effects of systemic administration of Tiludronate (TIL) on ligature-induced periodontitis in rats.

Methods: 32 adult male rats were divided into four groups (n=8): C, PD, PD-TIL5, PD-TIL15 (C–Control group, PD–Periodontitis groups). On PD groups, a ligature was placed in the cervical area of the right mandibular 1st molar of each rat. After 15 days, TIL solutions at dosages of 5 mg/kg (group PD-TIL5) or 15 mg/kg (group PD-TIL15) were subcutaneously administered 5 times a week for 3 weeks. The animals were euthanized at the 36th day. Histometric, using image analysis software, and histologic analyses were performed. Data were statistically analyzed (ANOVA, Tukey, $p<0.05$).

Results: In the area between 1st and 2nd molars, alveolar bone loss was significantly reduced in group PD-TIL5 (1.12mm±0.24), when compared with groups PD (1.70mm±0.32, $p<0.01$) and PD-TIL15 (1.47mm±0.21, $p<0.05$). The animals from all PD groups presented more periodontal attachment loss than the ones from group C (0.12mm±0.09). There were no differences in periodontal attachment loss among PD groups (PD:0.53mm±0.19; PD-TIL5:0.37mm±0.09; PD-TIL15:0.52 mm±0.13; $p>0.05$).

Conclusions: Within the limits of this study, it can be concluded that (i) systemically-administered TIL solution (5 mg/kg body weight) reduced alveolar bone loss in established periodontitis in rats and (ii) the dosage of TIL may influence its anti-inflammatory and anti-resorptive properties

Keywords: Bisphosphonates; Periodontal disease; Bone; Resorption.

INTRODUCTION

The presence of a periodontopathogenic dental biofilm can induce an inflammatory reaction in periodontal tissues, leading to the secretion of proinflammatory cytokines, such as Interleukin-1β (IL-1β) and Tumor Necrosis Factor-α (TNF-α), prostaglandins and Matrix Metalloproteinases (MMPs) by immune cells (leucocytes and macrophages) and gingival fibroblasts[1, 2]. These inflammatory mediators stimulate the alveolar bone resorption performed by osteoclasts and also the apical migration of the junctional epithelium, characterizing the periodontitis. The severity and progression of this disease are modified in genetically susceptible individuals and/or in the presence of immunoderegulating risk factors, like diabetes mellitus and smoking[3]. For these susceptible patients, the major part of periodontal destruction may occur due to the inflammatory response of the host[4].

The prerequisites for a successful conventional periodontal treatment are the patient's cooperation, an adequate oral hygiene[5] and the mechanical debridement of tooth surfaces[6, 7]. Recently, a new approach for periodontal treatment, comprising the control of the host response to bacterial aggression, has been studied[4]. Bisphosphonates (BPs) are synthetic chemical drugs very efficient in the treatment of some bone diseases, such as osteoporosis, Paget's disease, multiple myeloma, hypercalcemia of malignancy and bone metastasis, decreasing the risk of fractures[8, 9]. The proven efficacy of BPs to inhibit the osteoclastic bone resorption[10] has led to their use in the management of periodontitis as a host modulating factor in the perspective of reducing the alveolar bone loss[11, 12].

Tiludronate (chloro-4-phenyl-thiomethylene-1,1-bisphosphonate), a bisphosphonate (BP) from the first generation, was characterized by dose-dependently inhibit bone resorption in several *in vivo* preclinical studies, including models of thyroparathyroidectomized[13], neurectomized[14] and ovariectomized[13] rats. *In vitro* studies demonstrated that this BP also presents anti-inflammatory actions as it can dose-dependently inhibit Interleukin-6 (IL-6) synthesis by osteoblasts[15] and the secretion of IL-1ß, IL-6, nitric oxide (NO) and TNF-α by activated macrophages[16]. The inhibitory effects of Tiludronate (TIL) on important enzymes for the degradation of extracellular matrix components in periodontitis (MMP-1 and MMP-3) was also shown in human periodontal ligament cells[17]. Since TIL is a non-nitrogen-containing-bisphosphonate, it does not present the adverse effects usually associated with nitrogen-containing-bisphosphonates, such as gastrointestinal disorders[18], ocular lesions[19], acute-phase reactions (flu-like symptoms and increase in the circulating levels of IL-1, TNF-α

and IFN-γ)[18] and osteonecrosis of the jaws[20-22]. TIL is a safe compound with an appreciable therapeutic margin[23, 24].

Due to its anti-resorptive, anti-inflammatory properties and safety, TIL seems to be an efficient BP for periodontal management. Recently, it was demonstrated that the local application of TIL in the palatal gingiva of rats with periodontitis led to a decrease in alveolar bone loss[25]. To the best of our knowledge, there are no studies evaluating the influence of systemic administration of TIL in periodontitis. In this context, the purpose of this study was to histologically analyze the effects of systemic administration of TIL on ligature-induced periodontitis in rats.

MATERIALS AND METHODS

Sample

This study was conducted in compliance with the ethical principles of animal experimentation, as well as standards for the didactic-scientific practice of vivisection and the Universal Declaration of Animal Rights by United Nations Educational, Scientific and Cultural Organization (UNESCO). The present study was conducted only after review and approval by the Ethics Committee on Animal Research at Federal University of Ceara - UFC (protocol 099/2011).

Experimental Model

Thirty-two, 3- to 4-month-old, male rats (*Rattus norvegicus, albinus,* Wistar), weighing between 300 and 350 g, were used (Central Animal Facility of the Federal University of Ceara-UFC). The rats were kept in a room with a 12-hour light/dark cycle and temperature between 22 and 24°C. Throughout the experiment, the animals were housed in plastic cages and fed with selected solid diet and water *ad libitum*. They were randomly assigned to one of four experimental groups (n = 8), according to the following protocol:

- Group C (Control): Periodontitis (PD) was not induced and there was no systemic administration of Tiludronate (TIL);
- Group PD: PD was induced with ligature and there was no systemic administration of TIL;

- Group PD-TIL 5: PD was induced with ligature and TIL solutions at dosages of 5 mg/kg were subcutaneously administered;
- Group PD-TIL 15: PD was induced with ligature and TIL solutions at dosages of 15 mg/kg were subcutaneously administered.

Induction of Periodontitis with Ligature

On day 1, all animals were anesthetized by an intramuscular injection of xylazine* (6 mg/kg body weight) and ketamine† (70 mg/kg body weight). After general anesthesia, the animals were placed on the operating table, which allowed keeping the rats mouth opened, facilitating the access to posterior teeth of the mandible. Except for the animals from group C, a cotton ligature was placed in the cervical area of the right mandibular 1st molar of each rat, remaining supragingivally at both buccal and lingual faces. The knot was positioned at the buccal face of the tooth.

After 15 days of periodontitis induction, all animals were anesthetized again and their oral cavities were observed. In animals from groups PD, PD-TIL 5 and PD-TIL 15, it was observed if the ligature was still in position. In groups PD-TIL 5 and PD-TIL 15, subcutaneous administrations of tiludronic acid‡ (TIL) solutions at dosages of 5 mg/kg body weight and 15 mg/kg body weight, respectively, were initiated. Subcutaneous injections were performed on the back of the animals, next to the cephalic area, five times a week, always in the same time, during 3 weeks. Throughout the experimental period, the animals were weighed daily and the dosages of the bisphosphonate TIL were adapted accordingly.

At the 36th day after periodontits induction, the animals were euthanized under anesthesia with a final solution of xylazine (30 mg/kg body weight) and ketamine (240 mg/kg body weight).

Histomorphometric Analysis

The right hemimandible was excised, fixed in 10% neutral formalin for 48 hours, rinsed with water and then decalcified in 4% Ethylenediamine tetraacetic acid (EDTA) solution. After complete decalcification, the specimens were processed and embedded in paraffin. Serial sections, 5 μm thick, were obtained in a mesiodistal direction. The sections were stained with hematoxylin and eosin (H&E) for analysis by light microscopy.

For histometric analysis, sections representing the most central buccal-lingual portion in the area between 1st and 2nd right mandibular molars were selected. The images of the histologic sections were captured by a digital camera§ connected to a light microscope‖ with an original magnification of x40. The digital images were saved on a computer and then copied to ‘‘Image J’’ software¶, which was used for histometric analysis. In order to assess the interproximal alveolar bone level (ABL), a line connecting the cementoenamel junction (CEJ) of the 1st molar to the CEJ of the 2nd molar was drawn. Then a second line, perpendicular to the first one, was drawn connecting the first line to the coronal portion of the interdental bone crest. Additionally, in order to measure the periodontal attachment level (PAL), a line connecting the CEJ of the 1st molar and the coronal portion of the junctional epithelium attached to this tooth was drawn. The histomorphometric analysis was performed by one calibrated examiner (N.P.R.F.) who was blinded to the experimental groups and treatments rendered.

Examiner Calibration

To estimate the intra and inter-examiner error, the histometric analysis was performed by two examiners who were blinded to the experimental groups and treatments rendered. A second sample was measured again 48 hours after the first measurement. The paired t test was used to calculate the intraexaminer error. A Pearson correlation analysis between the data obtained by the two examiners was also performed. P values > 0.05 in paired t test and r> 0.90 values in the Pearson correlation test were considered to estimate the feasibility of the proposed method.

Statistical Analysis

The data obtained in the histometric analysis were grouped and presented as means and standard deviations. The significance of differences among groups for ABL and PAL was verified by analysis of variance (ANOVA) followed by *post-hoc* Tukey test. The significance level was set at 5% in all tests.

RESULTS

Clinical Follow-Up

All animals tolerated the experimental procedures well and remained healthy throughout the experimental period. After 16 days of the induction of periodontitis, animals from group PD-TIL 15 presented more body weight gain than animals from groups C and PD (ANOVA, $p < 0.05$). On the other days, no significant differences regarding body weight variation were observed among groups ($p > 0.05$) (Figure 1).

Qualitative Histologic Analysis

The periodontal tissues in the interproximal area between 1st and 2nd molars were healthy and there was no periodontal attachment loss in group C (Figure 2A). All groups with induction of periodontitis (PD, PD-TIL 5 and PD-TIL 15) presented loss of the interdental papillae and apical migration of the junctional epithelium (Figures 2B, 2C, 2D). In these groups, disorganization and disruption of transseptal collagen fibers, presence of moderate mononuclear inflammatory infiltrate in subepithelial connective tissue and in the margins of the periodontal ligament, cementum loss, interdental alveolar bone resorption and root resorption were also observed. Overall, group PD-TIL 5 showed less severe signs of periodontal inflammation, with an inflammatory infiltrate restricted to the coronal portion of the gingival margin, gingival fibers more organized and less tissue destruction than the other groups with periodontitis.

Examiner Calibration

There were no significant differences between the histometric measurements performed by the same examiner when the first and the second evaluations were compared ($p>0.05$). There was also a significant correlation between the measurements obtained by the two examiners ($r>0.90$).

Histometric and Statistical Analyses

The animals from group PD (1.70 mm ± 0.32) presented alveolar bone loss significantly greater than the animals from group C (1.24 mm ± 0.20, $p < 0.05$). Alveolar bone loss was significantly reduced in group PD-TIL 5 (1.12 mm ± 0.24), when compared with groups PD ($p < 0.01$) and PD-TIL 15 (1.47 mm ± 0.21, $p < 0.05$).

In relation to the periodontal attachment levels, the animals from all groups with induction of periodontitis (PD, PD-TIL 5 and PD-TIL 15) presented periodontal attachment loss significantly greater than the ones from group C (0.12 mm ± 0.09). Although group PD-TIL 5 (0.37 mm ± 0.09) has presented less periodontal attachment loss than groups PD (0.53 mm ± 0.19) and PD-TIL 15 (0.52 mm ± 0.13), these differences were not statistically significant ($p>0.05$).

Data obtained from histometric analysis and the comparisons among groups are depicted in Figures 3 and 4.

DISCUSSION

Based on the current knowledge about the mechanisms of action of bisphosphonates (BPs), their use in periodontal research shows a promising method of managing periodontal diseases by modifying the host response[4]. Recently, we observed that local administration of Tiludronate (TIL) reduced alveolar bone loss during the development of ligature-induced periodontitis in rats[25]. In this context, the purpose of the present study was to evaluate the effects of systemic administration of TIL on established periodontitis in rats.

The model of experimental periodontitis used in this study, already reported previously[26-28], allowed a successful induction of the disease. In the animals of group PD, which received the ligature, alveolar bone loss, periodontal attachment loss and moderate inflammatory infiltrate were observed. These findings were not detected or were slighter in the animals from Control group, which did not receive any intervention.

Several studies have reported that systemic administrations of BPs were effective in reducing alveolar bone loss and/or the expression of inflammatory mediators in experimental periodontitis[29-33]. However, these effects were evaluated at short periods of time after induction of periodontitis in these studies. As mentioned by Goya et al[34], it is important to use an experimental model of chronic periodontitis to evaluate whether BPs would also reduce bone resorption in chronic disease progression. To the best of our knowledge, before the present study, only the ones by Tani-Ishii et al[35] and Cetinkaya et al[36], using incadronate and risedronate, respectively, used this model of experimental periodontitis. Three weeks in the rat correspond to approximately 1 year in humans[37]. The use of BPs for this length of time was reported as a therapeutic treatment duration for periodontitis in a clinical trial[11].

The bisphosphonate (BP) TIL was chosen for its effects on bone metabolism[13, 14, 23, 38] and anti-inflammatory properties[15, 16]. Calcified tissues appear to be the main target for

deposition of this drug[39]. TIL inhibits IL-6 synthesis by osteoblasts[15] and the secretion of IL-1ß, IL-6, NO and TNF-α by activated macrophages[40]. Furthermore, studies have shown that TIL is capable of increasing bone mineral density[41, 42]. Other factor that favors the choice for this BP is that TIL presents a long skeletal retention time, leading to a marked persistent biological effect[43].

In this study, the anti-resorptive action of TIL was confirmed, since the animals that received TIL solutions at the dosage of 5 mg/kg body weight (group PD-TIL 5) presented less alveolar bone loss than the animals from group PD. The alveolar bone level of group PD-TIL 5 did not differ from the animals of the Control group, without the periodontitis induction. The bone loss observed in control animals may be explained by the physiological bone remodeling process that occurs with time and was already expected in a study with an extended duration[44].

Only the administration of TIL solutions at 5 mg/kg provided a significant decrease in alveolar bone loss in relation to the group PD. The animals that received the dosage of 15 mg/kg presented more alveolar bone loss than the animals which received the smaller dosage, showing a dose-dependent effect of the drug. It was already observed in other studies[13-16, 23], including a previous study from our group with local administration of TIL in rats with ligature-induced periodontitis (data not published). In this previous study, the histometric analysis revealed that the dosages of 0.1 and 0.3 mg/kg body weight did not lead to a significant reduction in alveolar bone loss, while it was demonstrated when the TIL at a dosage of 1 mg/kg was administered.

In fact, it seems that the different effects of the BPs on periodontitis depend on the type of the BP used[45], according to their side chains[46], on the duration of administration[36, 45] and also on the dosage[45]. Brunsvold et al. (1992)[47] and Weinreb et al. (1994)[48] assessed the effect of alendronate using a model of experimental periodontitis in monkeys, induced by ligature and subsequently inoculating with microorganisms. Both studies concluded that systemic administration of a 0.05 mg/kg dose of alendronate delays the progression of periodontitis, although higher doses do not. These results, in conjunction with the results of the present study, suggest that some BPs in low dosages may be more efficient in inhibiting alveolar bone resorption than higher dosages.

Regarding the periodontal attachment levels, all groups presented greater attachment losses when compared with the Control group and there were no differences among the groups with periodontitis induction. Even though there were no significant differences

between groups PD-TIL 5 and PD-TIL 15, there was a trend toward decreased attachment loss in group PD-TIL 5, corroborating a dose-dependent effect of the TIL, as mentioned before.

As the mechanical control of the dental biofilm (mainly scaling and root planing) represents the conventional periodontal treatment currently, it is necessary to investigate whether the administration of TIL would provide additional improvements. The applicability of BPs for periodontal diseases may increase in those individuals in whom conventional periodontal therapy is not convenient, such as medically compromised, physically and mentally challenged, or elderly patients[36]. Further studies are required also to generate dose-response curves and evaluate different therapeutic regimens, since only one regimen was analyzed in the present study (5 times a week, during 3 weeks). Since BPs are known to bind strongly to hydroxyapatite and to remain in bone tissue for long periods of time[49], it is possible to obtain an effect of the same magnitude by administering the drugs less frequently and/or for a shorter duration[50].

CONCLUSIONS

Within the limits of this study, it can be concluded that administration of TIL at a dosage of 5 mg/kg, during 3 weeks, reduced alveolar bone loss in established periodontitis in rats. Furthermore, the dosage of TIL may influence its anti-inflammatory and anti-resorptive properties.

FOOTNOTES

*Rompum®, Bayer Saúde Animal, São Paulo, SP, Brazil

†Dopalen®, Agribands, Paulínia, SP, Brazil

‡Tildren®, Ceva Saúde Animal Ltda., Paulínia, SP, Brazil

§C-SHG, Nikon Digital Sight DS-2MV, Tokio, Japan

‖Eclipse E200 MVR, Nikon, Tokio, Japan

¶National Institutes of Health, Washington, DC, USA

ACKN
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FIGURE LEGENDS

Figure 1. Body weight variation (g) through the experimental period (ANOVA, $p<0.05$, *compared with groups C and PD). C-Control; PD-Periodontitis; TIL-Tiludronate.

Figure 2. Photomicrographs of the periodontal tissues in the interdental area between 1st and 2nd molars. **A)** group C; **B)** group PD; **C)** group PD-TIL 5; **D)** group PD-TIL 15. H&E, original magnification x40. C-Control; PD-Periodontitis; TIL-Tiludronate.

Figure 3. Means and standard deviations of the alveolar bone level (mm), with comparisons among groups (ANOVA, Tukey, *$p<0.05$, **$p<0.01$). C-Control; PD-Periodontitis; TIL-Tiludronate.

Figure 4. Means and standard deviations of the periodontal attachment level (mm), with comparisons among groups (ANOVA, Tukey, *$p<0.05$, **$p<0.001$). C-Control; PD-Periodontitis; TIL-Tiludronate.

Figure 1.

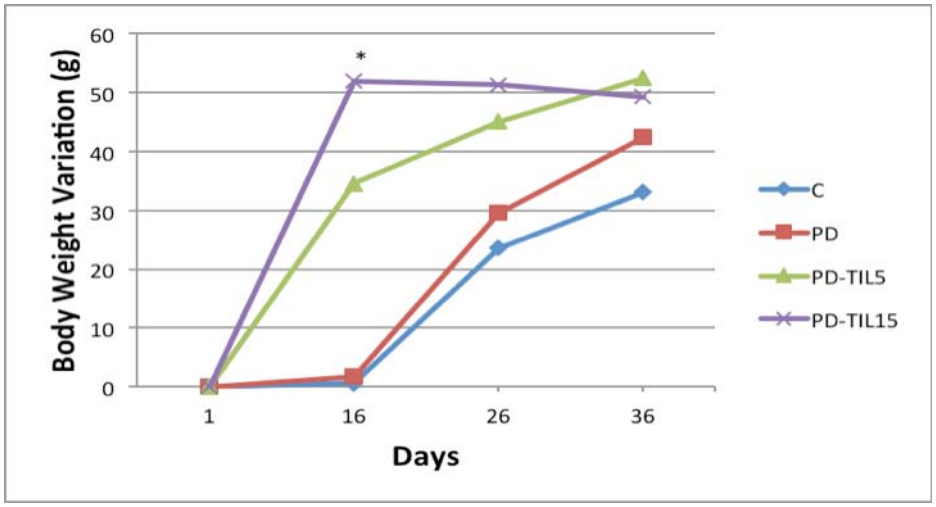


Figure 2.

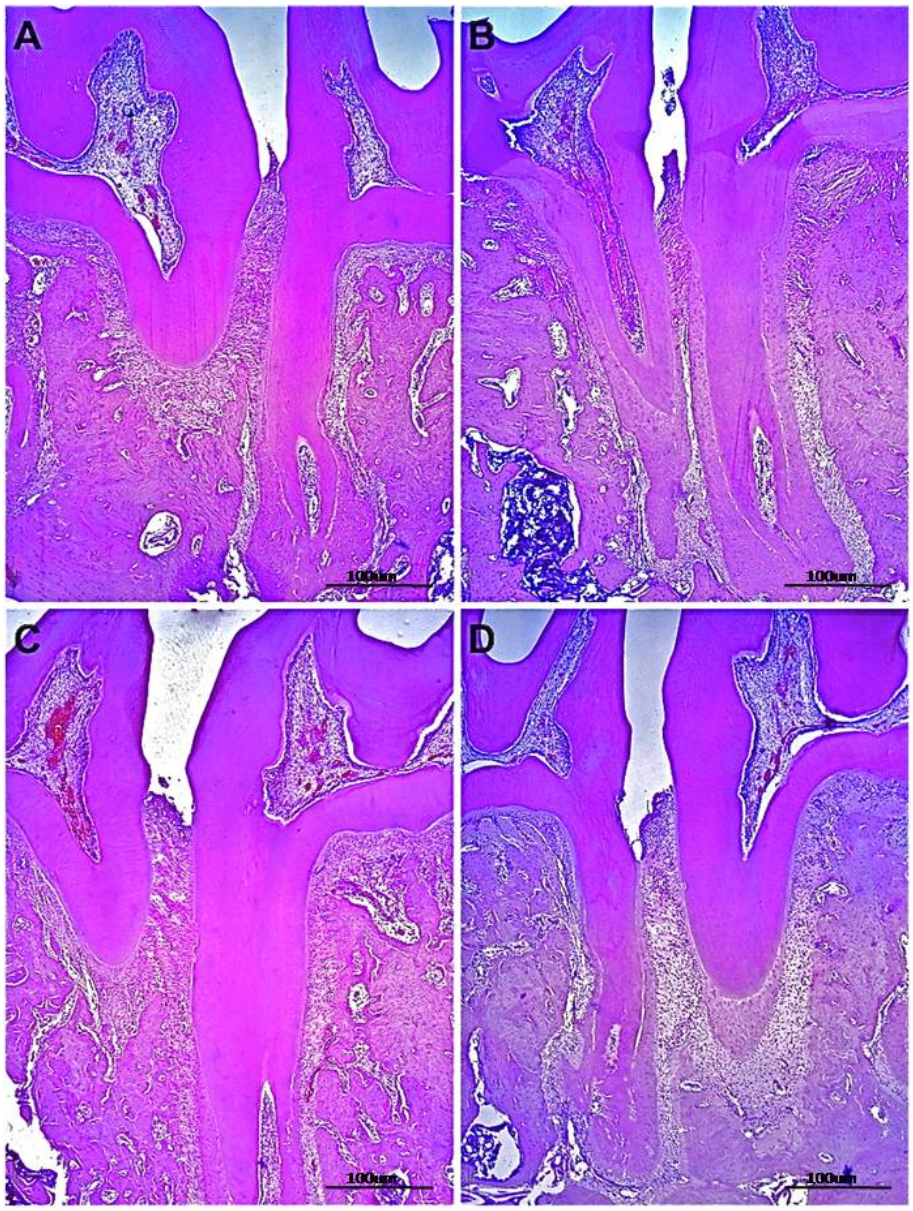


Figure 3.

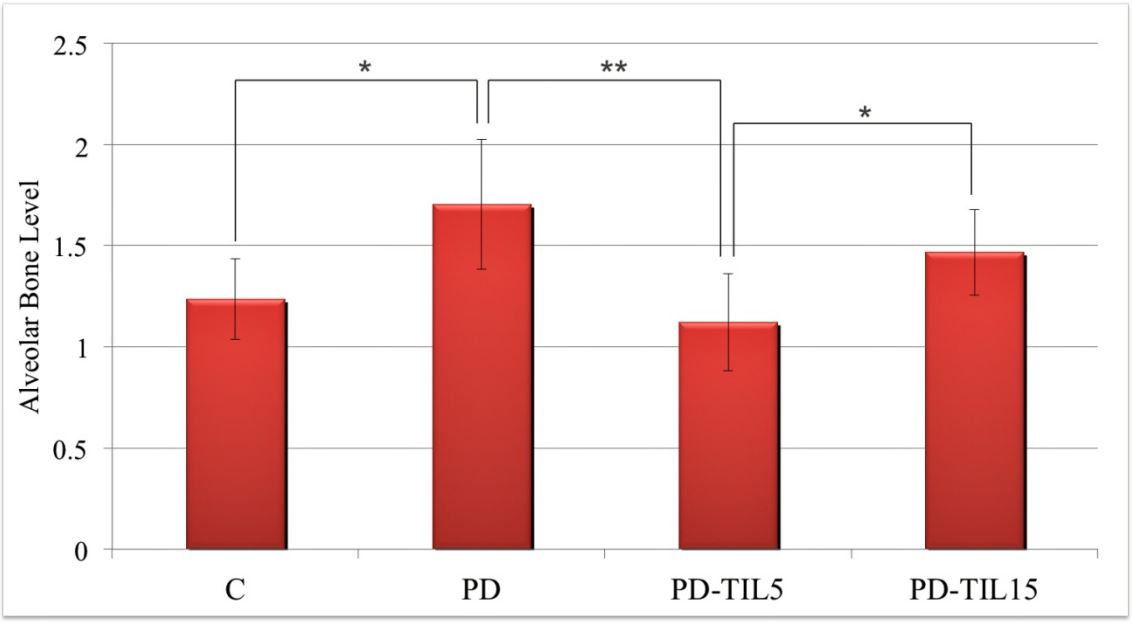
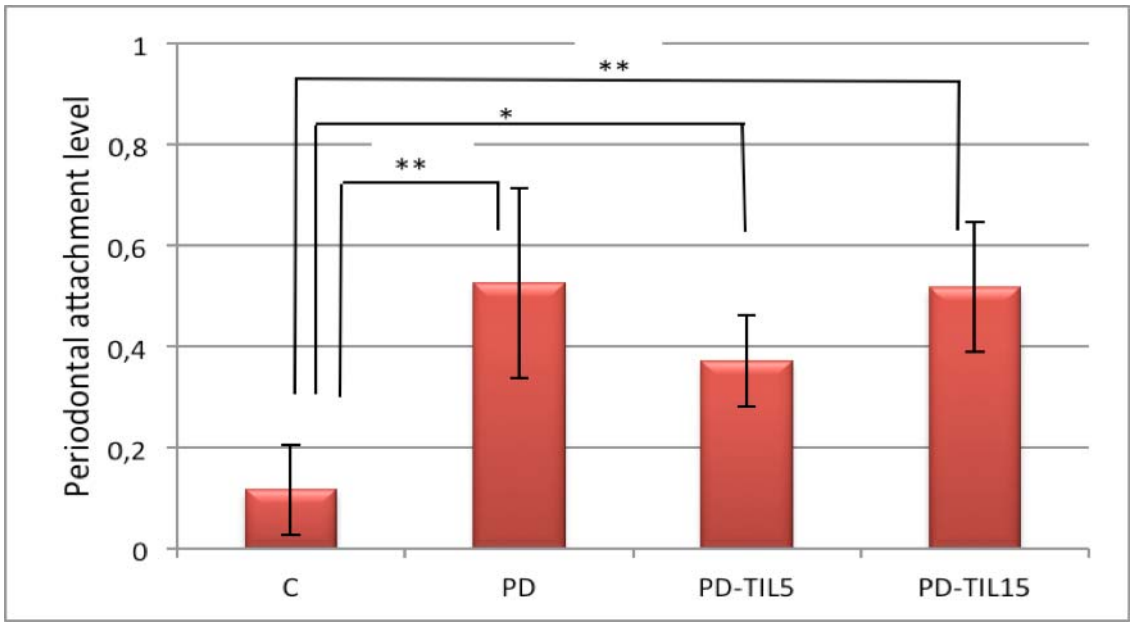


Figure 4.



CAPÍTULO 2:

"Effects of Tiludronate Administration as an Adjunctive Therapy on Experimental Periodontitis in Rats."

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Running title: Tiludronate as an adjunctive to mechanical periodontal treatment.

Summary Sentence: The adjunctive therapy with Tiludronate provides no additional benefit over mechanical periodontal treatment in experimental periodontitis.

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ABSTRACT

Background: Scaling and Root Planing (SRP) has been a key to periodontitis treatment. However, bisphosphonates has been investigated as adjunctive therapy in periodontitis. The purpose of this study was to histologically analyze the effects of systemic administration of Tiludronate (TIL) as an adjunctive therapy to mechanical periodontal treatment on ligature-induced periodontitis in rats.

Methods:40 rats were divided into five groups (n=8): C,PD,PDT,PDT-TIL5,PDT-TIL15 (C–Control group, PD–Periodontitis groups). On PD groups, a ligature was placed in the cervical area of the right mandibular 1st molar. After 15 days, ligatures from groups PDT, PDT-TIL5 and PDT-TIL15 were removed and SRP was performed. TIL solutions at 5 mg/kg (PDT-TIL5) or 15 mg/kg (PDT-TIL15) were subcutaneously administered 5 times a week for 3 weeks. The animals were euthanized at the 36th day. Histometric and histologic analyses were performed. Data were statistically analyzed (ANOVA,Tukey,$p<0.05$).

Results:There were no differences ($p>0.05$) in alveolar bone losses among groups PDT (1.27mm±0.15), PDT-TIL5 (1.18mm±0.10) and PDT-TIL15 (1.26mm±0.40). The alveolar bone losses found in these groups were slighter than the alveolar bone loss observed in group PD and did not statistically differ from the alveolar bone loss found in group C. Animals from all groups with periodontitis induction (group PD:0.59 mm±0.16;group PDT:0.39mm±0.07;group PDT-TIL5:0.42mm±0.05;group PDT-TIL15:0.48mm±0.09) presented periodontal attachment losses statistically greater than the animals from group C (0.12 mm±0.09,$p<0.001$). Groups PDT and PDT-TIL5 presented less periodontal attachment loss than group PD ($p<0.05$).

Conclusion: It can be concluded that systemically-administered TIL provided no additional benefit over mechanical periodontal therapy in rats with experimental periodontitis.

Keywords: Bisphosphonates; Periodontal disease; Bone; Resorption; Scaling and Root Planing.

INTRODUCTION

The prerequisites for a successful conventional periodontal treatment are the patient's cooperation, an adequate oral hygiene[1, 2] and the mechanical debridement of tooth surfaces[3, 4]. In genetically susceptible individuals and/or in the presence of immunoderegulating risk factors, like diabetes mellitus and smoking, the severity and progression of periodontitis are modified[5], what may hardens the control of the disease. The recognized importance of the host inflammatory response in the pathogenesis of periodontal diseases presents the opportunity to explore new treatment strategies[6].

Bisphosphonates (BPs) are synthetic chemical drugs very efficient in the treatment of some bone diseases, such as osteoporosis, Paget's disease, multiple myeloma, hypercalcemia of malignancy and bone metastasis, decreasing the risk of fractures[7, 8]. The proven efficacy of BPs to inhibit the osteoclastic bone resorption[9] has led to their use in the management of periodontitis as a host modulating factor, in the perspective of reducing alveolar bone loss[10, 11]. Some BPs also presented antibacterial activity[12, 13] and anti-inflammatory effects on experimental periodontitis[12, 14, 15]. Because of these actions, BPs seems to be a promising alternative for periodontal treatment and have been evaluated in animal and clinical studies[11, 12, 16].

Tiludronate (chloro-4-phenyl-thiomethylene-1,1-bisphosphonate), a bisphosphonate (BP) from the first generation, was characterized by dose-dependently inhibit bone resorption in several *in vivo* preclinical studies, including models of thyroparathyroidectomized[17], neurectomized[18] and ovariectomized[17] rats. *In vitro* studies demonstrated that this BP also presents anti-inflammatory actions as it can dose-dependently inhibit Interleukin-6 (IL-6) synthesis by osteoblasts[19] and the secretion of IL-1ß, IL-6, nitric oxide (NO) and TNF-α by activated macrophages[20]. The inhibitory effects of Tiludronate (TIL) on important enzymes for the degradation of extracellular matrix components in periodontitis (MMP-1 and MMP-3) were also shown in human periodontal ligament cells[21]. Since TIL is a non-nitrogen-containing-bisphosphonate, it does not present the adverse effects usually associated with nitrogen-containing-bisphosphonates, such as gastrointestinal disorders[22], ocular lesions[23], acute-phase reactions (flu-like symptoms and increase in the circulating levels of IL-1, TNF-α and IFN-γ)[22] and osteonecrosis of the jaws[2, 24, 25]. TIL is a safe compound with an appreciable therapeutic margin[26, 27]. Due to its anti-resorptive, anti-inflammatory properties and safety, TIL seems to be an efficient BP for periodontal management.

Recently, it was demonstrated that the local application of TIL in the palatal gingiva of rats with periodontitis decreased alveolar bone loss[28]. Our group also observed that systemic administrations of TIL reduced alveolar bone loss in established periodontitis in rats (data not published). To the best of our knowledge, there are no histologic studies evaluating the effects of BPs administration as an adjunctive to mechanical periodontal therapy. The purpose of this study was to histologically analyze the effects of systemic administration of TIL as an adjunctive therapy to mechanical periodontal treatment on ligature-induced periodontitis in rats.

MATERIALS AND METHODS

Sample

This study was conducted in compliance with the ethical principles of animal experimentation, as well as standards for the didactic-scientific practice of vivisection and the Universal Declaration of Animal Rights by United Nations Educational, Scientific and Cultural Organization (UNESCO). The present study was conducted only after review and approval by the Ethics Committee on Animal Research at Federal University of Ceara - UFC (protocol 099/2011).

Experimental Model

Forty, 3- to 4-month-old, male rats (*Rattus norvegicus, albinus,* Wistar), weighing between 300 and 350 g, were used (Central Animal Facility of the Federal University of Ceara-UFC). The rats were kept in a room with a 12-hour light/dark cycle and temperature between 22 and 24°C. Throughout the experiment, the animals were housed in plastic cages and fed with selected solid diet and water *ad libitum*. They were randomly assigned to one of five experimental groups (n = 8), according to the following protocol:

- Group C (Control): Periodontitis (PD) was not induced and there was no systemic administration of Tiludronate (TIL);
- Group PD: PD was induced with ligature and there was no systemic administration of TIL;

- Group PDT: PD was induced with ligature. After 15 days, ligature was removed and Scaling and Root Planing (SRP) was performed. There was no systemic administration of TIL;
- Group PDT-TIL 5: PD was induced with ligature. After 15 days, ligature was removed, SRP was performed and subcutaneous administrations of TIL solutions at dosages of 5 mg/kg were started;
- Group PDT-TIL 15: PD was induced with ligature. After 15 days, ligature was removed, SRP was performed and subcutaneous administrations of TIL solutions at dosages of 15 mg/kg were started.

Induction of Periodontitis with Ligature

On day 1, all animals were anesthetized by an intramuscular injection of xylazine* (6 mg/kg body weight) and ketamine† (70 mg/kg body weight). After general anesthesia, the animals were placed on the operating table, which allowed keeping the rats mouth opened, facilitating the access to posterior teeth of the mandible. Except for the animals from group C, a cotton ligature was placed in the cervical area of the right mandibular 1st molar of each rat, remaining supragingivally at both buccal and lingual faces. The knot was positioned at the buccal face of the tooth.

After 15 days of periodontitis induction, all animals were anesthetized again and their oral cavities were observed. In animals from groups PDT, PDT-TIL5 and PDT-TIL15, the ligatures were removed and their right mandibular 1st molars were submitted to SRP with Mini Five 13- 14 curettes‡, through 10 distal-mesial traction movements in both buccal and lingual aspects of the teeth. The furcation and interproximal areas were scaled with the same curettes through cervical-occlusal traction movements. SRP was performed by the same experienced and blinded operator. In groups PDT-TIL 5 and PDT-TIL 15, subcutaneous administrations of tiludronic acid§ (TIL) solutions at dosages of 5 mg/kg body weight and 15 mg/kg body weight, respectively, were initiated. Subcutaneous injections were performed on the back of the animals, next to the cephalic area, five times a week, always in the same time, during 3 weeks. Throughout the experimental period, the animals were weighed daily and the dosages of the bisphosphonate were adapted accordingly.

At the 36th day after periodontits induction, the animals were euthanized under anesthesia with a final solution of xylazine (30 mg/kg body weight) and ketamine (240 mg/kg body weight).

Histomorphometric Analysis

The right hemimandible was excised, fixed in 10% neutral formalin for 48 hours, rinsed with water and then decalcified in 4% Ethylenediamine tetraacetic acid (EDTA) solution. After complete decalcification, the specimens were processed and embedded in paraffin. Serial sections, 5 μm thick, were obtained in a mesiodistal direction. The sections were stained with hematoxylin and eosin (H&E) for analysis by light microscopy.

For histometric analysis, sections representing the most central buccal-lingual portion in the area between 1st and 2nd right mandibular molars were selected. The images of the histologic sections were captured by a digital camera‖ connected to a light microscope¶ with an original magnification of x40. The digital images were saved on a computer and then copied to ‘‘Image J’’ software#, which was used for histometric analysis. In order to assess the interproximal alveolar bone level (ABL), a line connecting the cementoenamel junction (CEJ) of the 1st molar to the CEJ of the 2nd molar was drawn. Then a second line, perpendicular to the first one, was drawn connecting the first line to the coronal portion of the interdental bone crest. Additionally, in order to measure the periodontal attachment level (PAL), a line connecting the CEJ of the 1st molar and the coronal portion of the junctional epithelium attached to this tooth was drawn. The histomorphometric analysis was performed by one calibrated examiner (N.P.R.F.) who was blinded to the experimental groups and treatments rendered.

Examiner Calibration

To estimate the intra and inter-examiner error, the histometric analysis was performed by two examiners who were blinded to the experimental groups and treatments rendered. A second sample was measured again 48 hours after the first measurement. The paired t test was used to calculate the intraexaminer error. A Pearson correlation analysis between the data obtained by the two examiners was also performed. P values > 0.05 in paired t test and r> 0.90 values in the Pearson correlation test were considered to estimate the feasibility of the proposed method.

Statistical Analysis

The data obtained in the histometric analysis were grouped and presented as means and standard deviations. The significance of differences among groups for ABL and PAL was verified by analysis of variance (ANOVA) followed by *post-hoc* Tukey test. The significance level was set at 5% in all tests.

RESULTS

Clinical Follow-Up

All animals tolerated the experimental procedures well and remained healthy during the period of the study. No significant differences regarding body weight variation were observed among groups throughout the experimental period (ANOVA, $p > 0.05$) (Figure 1).

Qualitative Histologic Analysis

It was observed that periodontal tissues in the interproximal area between 1st and 2nd molars remained healthy and there was no periodontal attachment loss in group C (Figure 2A). All groups with induction of periodontitis (PD, PDT, PDT-TIL 5 and PDT-TIL 15) presented loss of the interdental papillae and apical migration of the junctional epithelium (Figures 2B, 2C, 2D, 2E). In group PD, disorganization and disruption of transseptal collagen fibers, presence of moderate mononuclear inflammatory infiltrate in subepithelial connective tissue and in the margins of the periodontal ligament, cementum loss, interdental alveolar bone resorption and root resorption were also observed. In groups PDT, PDT-TIL 5 and PDT-TIL 15, the signs of tissue destruction were less severe. Evidences of periodontal repair, with reorganization of the collagen fibers of the gingival connective tissue, were observed in these groups.

Examiner Calibration

There were no significant differences between the histometric measurements performed by the same examiner when the first and the second evaluations were compared ($p > 0.05$). There was also a significant correlation between the measurements obtained by the two examiners ($r > 0.90$).

Histometric and Statistical Analyses

Group PD (1.80 mm ± 0.23) presented alveolar bone loss significantly greater than group C (1.17 mm ± 0.15, $p < 0.01$). There were no differences ($p>0.05$) in alveolar bone losses among groups PDT (1.27 mm ± 0.15), PDT-TIL 5 (1.18 mm ± 0.10) and PDT-TIL 15 (1.26 mm ± 0.40). The alveolar bone losses found in these groups (PDT, PDT-TIL 5 and PDT-TIL 15) were slighter than the alveolar bone loss observed in group PD and did not statistically differ from the alveolar bone loss found in group C.

Regards the periodontal attachment level, the animals from all groups with periodontitis induction (group PD: 0.59 mm ± 0.16; group PDT: 0.39 mm ± 0.07; group PDT-TIL 5: 0.42 mm ± 0.05; group PDT-TIL 15: 0.48 mm ± 0.09) presented periodontal attachment losses statistically greater than the animals from group C (0.12 mm ± 0.09, $p < 0.001$). Groups PDT and PDT-TIL 5 presented less periodontal attachment loss than group PD ($p < 0.05$). However, the periodontal attachment losses presented by groups PDT, PDT-TIL 5 and PDT-TIL 15 were not statistically different ($p>0.05$).

Data obtained from histometric analysis and the comparisons among groups are depicted in Figures 3 and 4.

DISCUSSION

Recently, it has been demonstrated that the local administration of the bisphosphonate (BP) Tiludronate (TIL) in the gingiva decreased alveolar bone loss during the development of experimental periodontitis in rats[28]. Furthermore, our group observed that, when systemically administrated, TIL reduced alveolar bone loss in established periodontitis in rats (data not published). In this context, the purpose of the present study was to evaluate the effects of systemic administration of TIL as an adjunctive therapy to mechanical periodontal treatment on periodontal tissues of rats with ligature-induced periodontitis.

The model of experimental periodontitis used in this study, already reported previously[29-31], allowed a successful induction of the disease. The animals of group PD, which received the ligature and no treatments were rendered, presented alveolar bone and periodontal attachment losses significantly greater than the animals of the Control group, which did not receive any intervention.

Several studies have reported that systemic administrations of bisphosphonates (BPs) were effective in reducing alveolar bone loss and/or the expression of inflammatory mediators

in experimental periodontitis[12, 14, 15, 32]. However, to the best of our knowledge, this is the first study to histologically evaluate the adjunctive effect of BPs to mechanical periodontal therapy in experimental periodontitis. The studies that evaluated the adjunctive effect of BPs in periodontitis were clinical trials that analyzed alendronate, risedronate and neridronate[10, 33-36].

The BP TIL was chosen for its effects on bone metabolism[17, 18, 26, 37] and anti-inflammatory properties[19, 20]. Calcified tissues appear to be the main target for deposition of this drug[38]. TIL inhibits IL-6 synthesis by osteoblasts[19] and the secretion of IL-1ß, IL-6, NO and TNF-α by macrophages[20]. Furthermore, studies have shown that TIL is capable of increasing bone mineral density[39, 40]. Other factor that favors the choice for this BP is that TIL presents a long skeletal retention time, leading to a marked persistent biological effect[41]. In this study in rats, the time length for TIL administration was 3 weeks, which corresponds to approximately 1 year in humans[42]. The use of BPs for this period of time was reported as a therapeutic treatment duration for periodontitis in a clinical trial[10].

Currently, the basic approach to periodontal infections remains the removal of supra- and subgingival bacterial deposits by Scaling and Root Planing (SRP)[43]. Nonsurgical mechanical periodontal treatment is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infections[44]. The applicability of adjunctive therapies may increase in those individuals in whom conventional periodontal therapy is not convenient, such as medically compromised, physically and mentally challenged, or elderly patients[45, 46].

In this study, the efficacy of the mechanical periodontal therapy was confirmed, since all the groups submitted to this therapy (groups PDT, PDT-TIL 5 and PDT-TIL 15) presented less alveolar bone and periodontal attachment losses than the animals that did not receive the therapy (group PD). However, administration of TIL did not provide additional improvements than the ones obtained with the mechanical therapy. In the animals subjected to the removal of ligatures, SRP and BP administration (groups PDT-TIL 5 and PDT-TIL 15), there were no significant reductions in alveolar bone and periodontal attachment losses, when compared to the animals that received the mechanical therapy alone (group PDT). These results seem to be comparable to the results from a clinical trial where the BP neridronate was used in generalized chronic periodontitis and did not result in additional improvements[36]. On the other hand, the results of the present study diverge from many other clinical trials which found that the adjunctive therapy with BPs improved the results of the non-surgical periodontal therapy, as verified by clinical and/or radiographic parameters[10, 33-35].

The lack of additional benefits of the adjunctive therapy with TIL in the present study may be related to the potential of repair of the animals used. Spolidorio et al[47] reported that, 15 days after the removal of molars' ligatures, rats showed a spontaneous periodontal healing, with a significant reduction in the distance between CEJ and alveolar bone crest. In the same way, the period of 3 weeks through which the animals remained without ligatures, in the present study, might have been enough for a repair of the periodontal tissues. Other hypothesis may be related to the limitations of our histomorphometric analysis for assessment of periodontal destruction and repair[47].

Since is known that TIL presents dose-dependent effects[17-20, 26], the results obtained in the present study may also be related to the dosage administered. Although we have found that the dosage of 5 mg/kg has been enough to reduce bone loss in a previous study (data not published), it is possible that it was not suitable to offer an extra benefit to that provided by the mechanical therapy alone. While there were no significant differences between the animals that received the dosages of 5 mg/kg and15 mg/kg in this study, there was a clear trend toward decreased periodontal attachment loss with the dosage of 5 mg/kg. In fact, group PDT-TIL 5 presented periodontal attachment loss significantly lower than the animals that did not receive mechanical treatment (group PD), whereas the group PDT-TIL 15 did not diverge from group PD.

Other interesting finding is that the bone loss presented by the animals from the groups subjected to mechanical periodontal treatment was not statistically different from the bone loss demonstrated by the animals without periodontits induction (Control group). The alveolar bone loss found in control animals may be explained by the growth and bone remodeling processes occurring in the jaw as a whole with time, in addition to rapid occlusal attrition of the teeth caused by the consumption/mastication of food, the consequence of which is movement of the molars in the occluso-disto-buccal direction[48-50].

Taking into consideration that most of the clinical trials[10, 33-35] demonstrated a positive effect of the adjunctive therapy with BPs in periodontitis management and also the limitations of the present study, further studies are needed to verify whether other dosages, therapeutic regimens and/or experimental models would present more favorable results. In addition, although the therapy with TIL has not shown additional benefits through the histometric analysis, it is possible that it has influenced levels of tissue density or the expression of inflammatory mediators involved in periodontitis.

CONCLUSION

Within the limits of this study, it can be concluded that systemically-administered TIL provided no additional benefit over mechanical periodontal therapy in rats with experimental periodontitis.

FOOTNOTES

* Rompum®, Bayer Saúde Animal, São Paulo, SP, Brazil
† Dopalen®, Agribands, Paulínia, SP, Brazil
‡ Hu-Friedy Co. Inc., Chicago, IL, USA
§ Tildren®, Ceva Saúde Animal Ltda., Paulínia, SP, Brazil
‖ C-SHG, Nikon Digital Sight DS-2MV, Tokio, Japan
¶ Eclipse E200 MVR, Nikon, Tokio, Japan
# National Institutes of Health, Washington, DC, USA

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FIGURE LEGENDS

Figure 1. Body weight variation (g) through the experimental period (ANOVA, $p>0.05$). C=Control; PD=Periodontitis; PDT=Periodontitis – Treatment; TIL=Tiludronate.

Figure 2. Photomicrographs of the periodontal tissues in the interdental area between 1st and 2nd molars. **A)** group C; **B)** group PD; **C)** group PDT; **D)** group PDT-TIL 5; group PDT-TIL 15. H&E, original magnification x40. C=Control; PD=Periodontitis; PDT=Periodontitis – Treatment; TIL=Tiludronate.

Figure 3. Means and standard deviations of the alveolar bone level (mm), with comparisons among groups (ANOVA, Tukey, *$p<0.01$, **$p<0.05$, ***$p<0.001$). C=Control; PD=Periodontitis; PDT=Periodontitis – Treatment; TIL=Tiludronate.

Figure 4. Means and standard deviations of the periodontal attachment level (mm), with comparisons among groups (ANOVA, Tukey, *$p<0.05$, **$p<0.001$). C=Control; PD=Periodontitis; PDT=Periodontitis – Treatment; TIL=Tiludronate.

Figure 1.

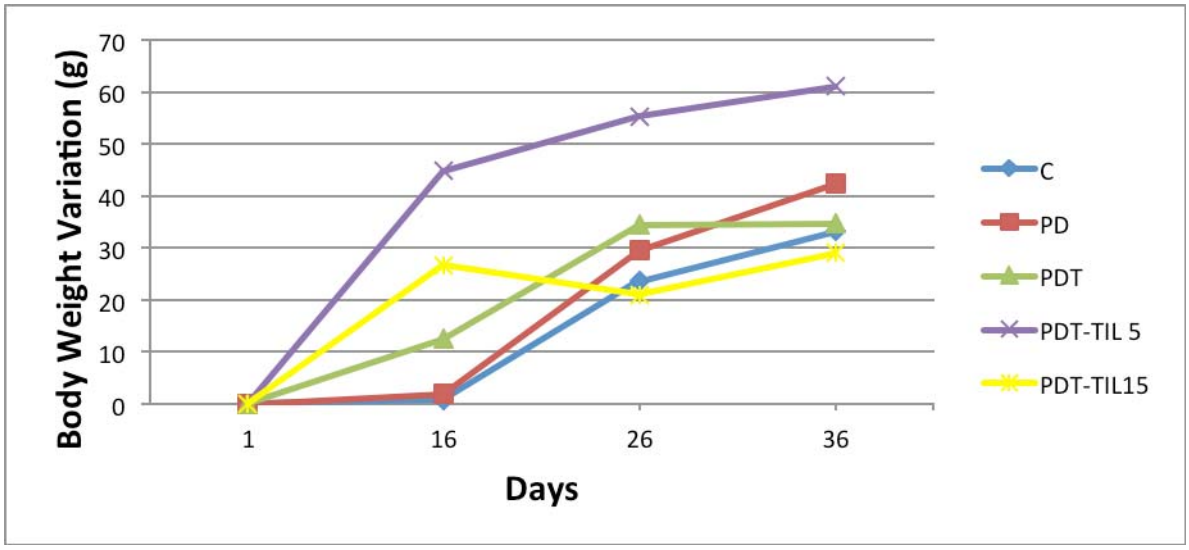


Figure 2.

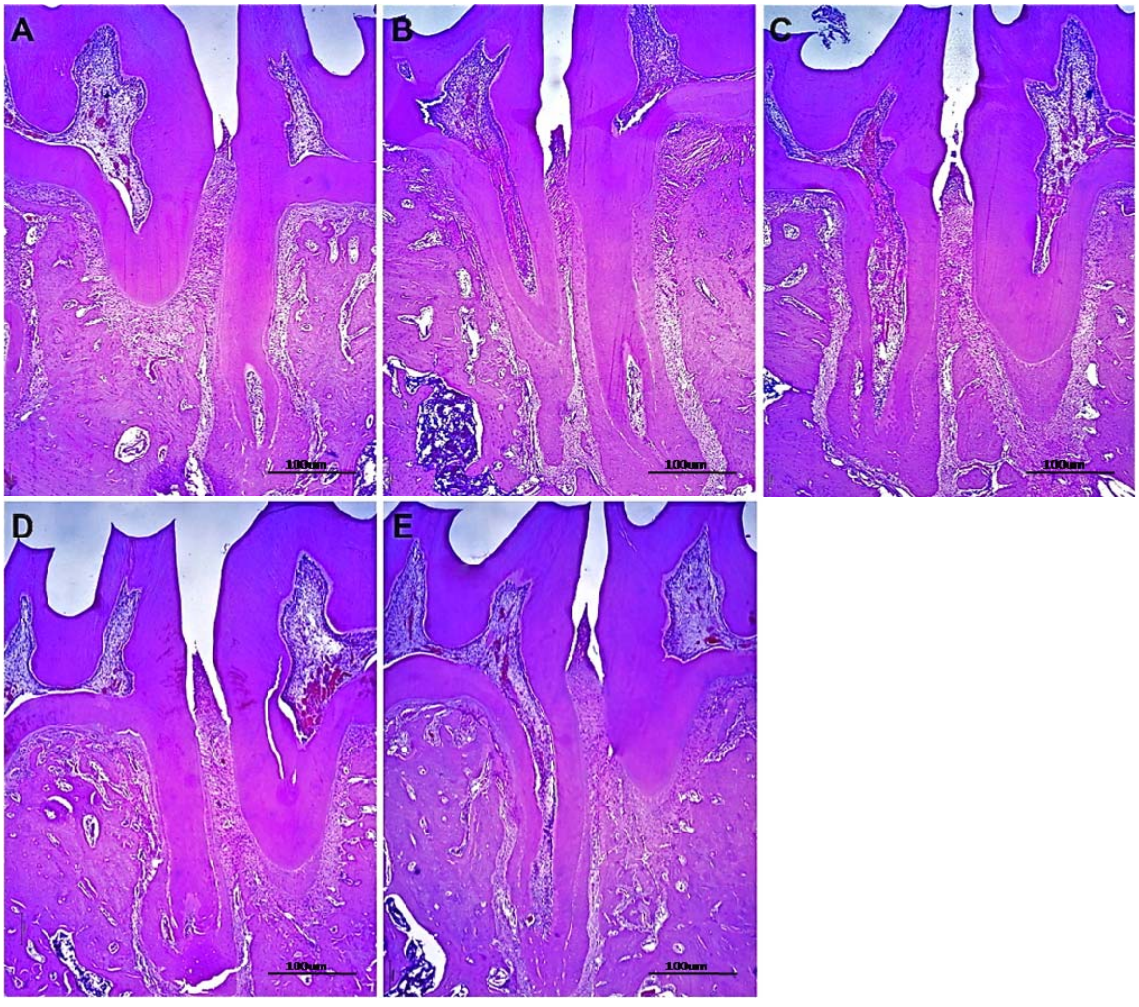


Figure 3.

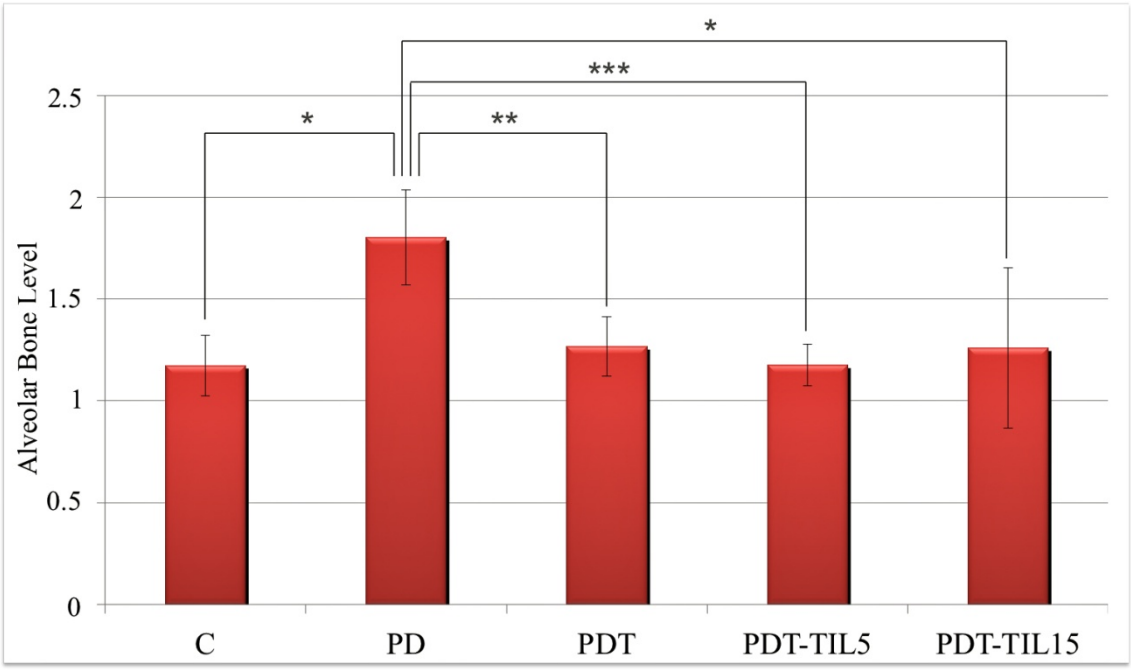
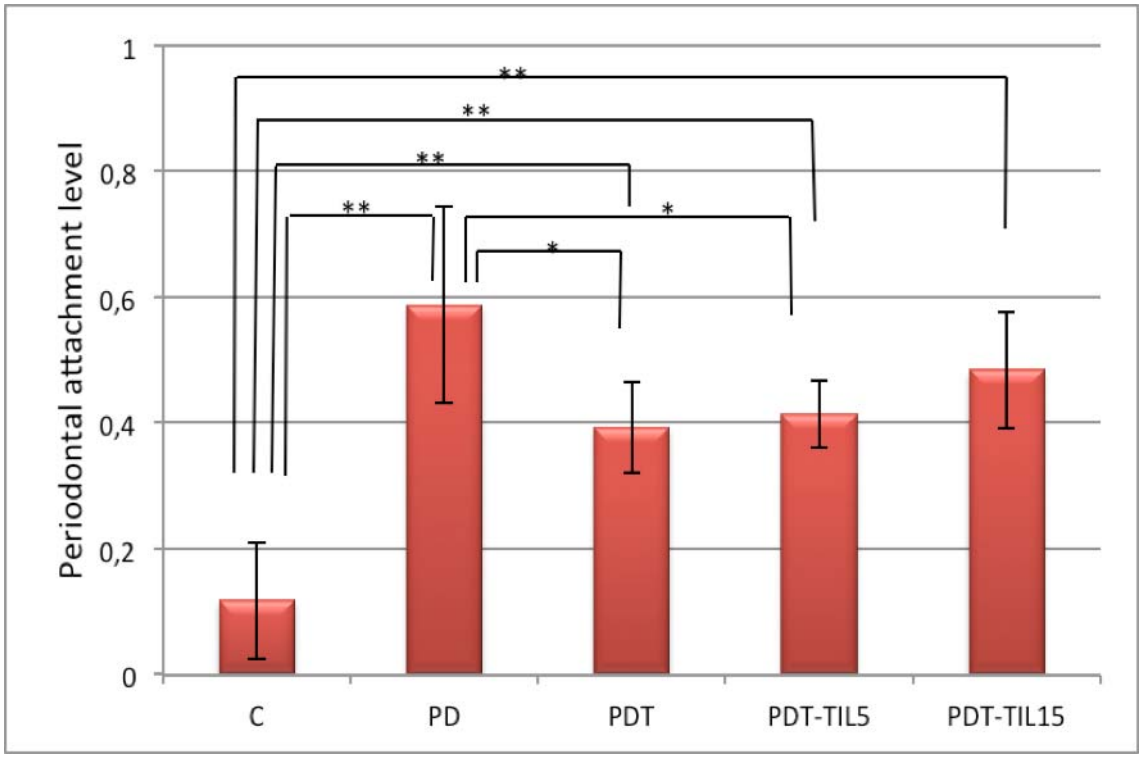


Figure 4.



4 CONCLUSÕES GERAIS

Dentro dos limites deste estudo, pode ser concluído que:

1. A administração de Tiludronato na dosagem de 5 mg/kg, durante 3 semanas, reduziu a perda óssea alveolar na periodontite estabelecida em ratos;
2. A dosagem do TIL pode influenciar suas propriedades antirreabsortivas e anti-inflamatórias;
3. A administração do Tiludronato não proporcionou benefícios adicionais à terapia periodontal mecânica em ratos com periodontite experimental.

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ANEXO A

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DECLARAÇÃO

Declaramos que o protocolo para uso de animais em experimentação nº 99/2011, sobre o projeto intitulado: **"AVALIAÇÃO DOS EFEITOS DA ADMINISTRAÇÃO SISTÊMICA DO BIFOSFANATO TILUDRONATO NA PERIODONTITE EXPERIMENTAL EM RATOS."**, de responsabilidade de FLÁVIA APARECIDA CHAVES FURLANETO, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA).

Declaramos ainda que o referido projeto foi aprovado pela Comissão de Ética em Pesquisa Animal – CEPA – em reunião realizada em 07 de dezembro de 2011.

Fortaleza, 12 de dezembro de 2011.

Profa. Dra. Nylane Maria Nunes de Alencar
Coordenadora da Comissão de Ética em Pesquisa Animal – CEPA

ANEXO B

DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas sujeitas a arbitragem, que constam da minha Dissertação de Mestrado, intitulada "**EFEITOS DA ADMINISTRAÇÃO DO BISFOSFONATO TILUDRONATO, ASSOCIADO OU NÃO À TERAPIA PERIODONTAL MECÂNICA, NA PERIODONTITE EXPERIMENTAL EM RATOS"** não infringem os dispositivos da Lei n.o 9.610/98, nem o direito autoral de qualquer editora.

Fortaleza, 28 de janeiro de 2013

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