

Effect of Simvastatin on induced apical periodontitis in rats: a tomographic and biochemical analysis

Efeito da Simvastatina sobre a periodontite apical induzida em ratos: análise radiográfica e bioquímica

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Resumo

Introdução: A simvastatina é uma das várias estatinas utilizadas no tratamento da hipercolesterolemia e, na odontologia, alguns estudos têm buscado relacioná-la ao reparo ósseo. **Objetivo:** Avaliar o efeito da simvastatina na progressão da periodontite apical induzida em ratos. **Material e método:** Para tanto, 36 ratos Wistar, previamente selecionados, foram divididos em 3 grupos (N=12). Grupo Controle (GC); Grupo Periodontite Apical (GPA); Grupo Periodontite Apical Associada à Simvastatina (GPAS). No primeiro dia do ensaio o GPA e o GPAS foram anestesiados e submetidos à abertura coronária do primeiro molar inferior direito. Durante trinta dias, o GPAS recebeu diariamente 6mg de simvastatina, através de gavagem. No trigésimo primeiro dia, todos os grupos foram submetidos à coleta de sangue e a eutanásia. As mandíbulas foram removidas e fixadas em formol. Para a mensuração das regiões periapicais, foram realizadas tomografias. Além disso, avaliou-se a massa corporal, glicemia e o lipidograma. Os dados foram submetidos à análise estatística ANOVA de uma via e teste Post Hoc de Tukey ($p < 0,05$). **Resultado:** Os resultados demonstram que o GPA ($3,42 \pm 0,65$) apresentou os maiores perímetros para espaço do ligamento periapical, seguido pelo GPAS ($1,54 \pm 0,78$) e GC ($0,64 \pm 0,24$), respectivamente ($p < 0,05$). Em relação ao lipidograma percebe-se o efeito da simvastatina avaliando-se a quantidade de glicose, triglicérides, HDL, VLDL ($p < 0,05$). Para a massa corporal o GPA foi o que mais ganhou peso ($264,75 \pm 44,11$); seguido pelo GC ($252,00 \pm 44,36$); e GPAS ($245,41 \pm 42,56$). Os três grupos apresentaram diferenças estatísticas entre si, de forma decrescente ($p < 0,05$). **Conclusão:** O uso da simvastatina diminuiu a progressão do aumento do espaço do ligamento periapical em ratos.

Descritores: Simvastatina; ratos; periodontite apical; endodontia.

Abstract

Introduction: Simvastatin is one of several statins that are used to treat hypercholesterolemia, and in dentistry, few studies have attempted to associate the administration of this compound with bone repair. **Objective:** To evaluate the effect of simvastatin on the progression of induced apical periodontitis in rats. **Material and method:** To this end, 36 male Wistar rats were divided into 3 groups (N=12): Induced Apical Periodontitis Associated with Simvastatin Group (APSG N=12), Induced Periodontitis Apical Induced Group (APG N=12) and Negative Control Group (CG). On the first day, APG and APSG were anesthetized, and the coronal opening of the mandibular first molar was performed. For thirty days, the APSG received 6 mg of simvastatin daily via gavage. On the thirty-first day, all groups underwent blood collection and euthanasia. The jaws were removed and fixed in formalin. CT scans were performed to measure the periapical regions. In addition, the body mass and lipid profile were analyzed. The data were subjected to statistical analysis (ANOVA and Tukey's test). **Result:** The APG (3.42 ± 0.65) showed the highest perimeters for the space periapical ligament, followed by APSG (1.54 ± 0.78) and CG (0.64 ± 0.24) ($p < 0.05$). The lipid profile revealed the effect of simvastatin on the amount of glucose, triglycerides, HDL, and VLDL ($p < 0.05$). Body mass APG showed the most weight gain (264.75 ± 44.11), followed by CG (252.00 ± 44.36) and APSG (245.41 ± 42.56). The three groups showed significant differences in decreasing order ($p < 0.05$). **Conclusion:** The use of simvastatin decreased the progression of the increasing periapical ligament space in rats.

Descriptors: Simvastatin; rats; periapical periodontitis; endodontics.

INTRODUCTION

Even with the advent of modern techniques for the prevention and treatment of many diseases, oral health is still considered a problem worldwide¹. The most common oral diseases include tooth decay and periodontitis^{1,2}. The focus on infection-induced biofilm in the oral cavity³ is an important element in the inflammatory response generated by the body⁴ and results in other problems associated with the well-being and health of the person⁵.

Simvastatin (SVT) is one of several statins used for the treatment of hypercholesterolemia, i.e., the bloodstream cholesterol levels that can cause serious damage to health, such as cardiovascular disease⁶.

The systemic mechanism of action of SVT has recently been explored⁷, but care should be taken because this drug has been shown to lead to the accumulation of cholesterol and chemical by-products. Despite the proven benefits, similar to most drugs, the prolonged use of SVT has been associated with side effects⁸.

In dentistry, several studies have attempted to associate SVT with repair and bone regeneration^{9,10}, which are urgently needed in the dental clinic, particularly when there is a need to use dental implants. Indeed, many studies have attempted to provide a better understanding of the mechanisms of SVT, which acts locally in the cascade of immuno-inflammatory diseases¹¹. Thus, the use of SVT as an adjunct in the treatment of marginal periodontitis has recently become a research field^{12,13}.

Studies concerning apical periodontitis induction are relevant¹⁴ and are being conducted to obtain a better understanding of the pathogenesis of this disease. The effect of simvastatin on induced apical periodontal diseases in rat has been proposed as a plausible^{15,16} contribution to research information that cannot be used in human subjects¹².

Thus, understanding the action of the systematic (oral) use of simvastatin in the periapical region is relevant and important, particularly when examined in an animal model to obtain relevant information on this subject^{14,16}. Thus, the objective of the present study was to evaluate the effects of simvastatin on induced apical periodontitis progression in rats.

MATERIAL AND METHOD

Initially, 36 male, adult Novegicus Wistar rats at 2 months of age, with an initial weight of approximately 210 grams, were selected for the Veterinary Hospital Vivarium at the University of Cuiabá – UNIC, Campus Beira Rio, Cuiabá MT. These animals underwent acclimation to the new environment for two weeks and were maintained in nine housing boxes (polyethylene 60 × 40 × 35) at four animals per cage, with standard diet and water ad libitum under a light/dark cycle of 12 hours, 23 °C and humidity 60% (CEUA - UNIC protocol 003/2014).

A technical assistant unaware of the research objectives in the three groups randomly distributed the animals into 3 groups: Induced Apical Periodontitis Associated with Simvastatin Group (APSG N=12); Induced Apical Periodontitis Group (APG N=12) and Negative Control Group (CG).

For apical periodontitis induction, all animals, except the CG group, received anesthesia via the intramuscular administration of 0.1 ml of ketamine hydrochloride (Dopalen, Agribrands, Animal Health, Paulinia, SP, Brazil) with 0.05 ml of xylazine hydrochloride (Rompun, Bayer, Animal Health, São Paulo, SP, Brazil) for every 100 grams of body weight¹⁷.

A round diamond bur was selected (1011-KG - Sorensen, Cotia, SP, Brazil), and it was adapted to a dental high speed rotation motor and used on the occlusal surface of the first lower right molar to pierce enamel and dentine and promote access to the pulp chamber. After surgery, a dose of dipyrone (2.5 mg for each 100 g of body weight) was intramuscularly injected into each animal from APG and APSG. The CG did not receive any intervention but were maintained in the same environment as the other groups.

After the induction of apical periodontitis, the animals APSG were administered a daily intake of six milligrams of simvastatin (Capsules with 6 mg, Natupharma - Handling of Pharmacy, Cuiabá- MT) using a gavage procedure via 10-ml syringe (BD Brazil, Curitiba-PR) coupled to a 10-cm cannula for injection (18G Scalp vein, Venescalp, Feira de Santana - BA, Brazil). The uninterrupted drug administration occurred for 30 days. The APG group underwent the same treatment procedure but with saline.

At 30 days after gavage, the animals were again anesthetized through an intramuscular injection of 0.1 ml of ketamine hydrochloride (Dopalen, Agribrands, Animal Health, Paulinia, SP, Brazil) with 0.05 ml of xylazine hydrochloride (Rompun Bayer, Animal Health, São Paulo, SP, Brazil) per 100 g of corporal weight¹⁷. Following the administration of anesthesia, the skin of the abdominal wall was incised diagonally at the abdomen base, forming a “V” after the displacement of the skin to the abdominal cavity. The internal organs were displaced to visualize the vena cava. Blood was collected by puncture of the vena cava using a 25 × 7 needle (Vacutainer - Becton Dickinson, Plymouth, UK) in a 5-ml tube.

Immediately at the end of each blood collection, euthanasia was performed by excess anesthesia. Immediately thereafter, the mandibles were removed and fixed in 10% formalin for 48 hours.

After fixation, the samples were scanned using a Kodak 9000 C3K, 60 KV, 5 MA tomographic apparatus. Initially, a sagittal plane of the apical region of each of the roots of the lower right first molar (Figures 1 and 2) through the axial acquisition protocol spacing between cuts of 0.76 mm with a pixel size of 0.332, resolution of 13,072 pixels/mm 60 kV and 20 mA, 10 cm FOV, and voxel size of 0.08 × 0.08 × 0.08 mm using the Kodak Dental Imaging Software program (3d module v 2.4 Kodak Dental Systems, Atlanta, GA, USA).

A single evaluator unaware of the research groups calibrated the perimeters of the lesions. The perimeters of each root lesions were summed and divided by the number of measured lesions, producing an average per animal.

For the weight parameters, the animals were weighed using a semi-analytical balance (BL 3200H, Shimadzu, Kyoto, Japan), and the weights were determined based on differences between the averages of the weights obtained at the beginning and end of the experiment.

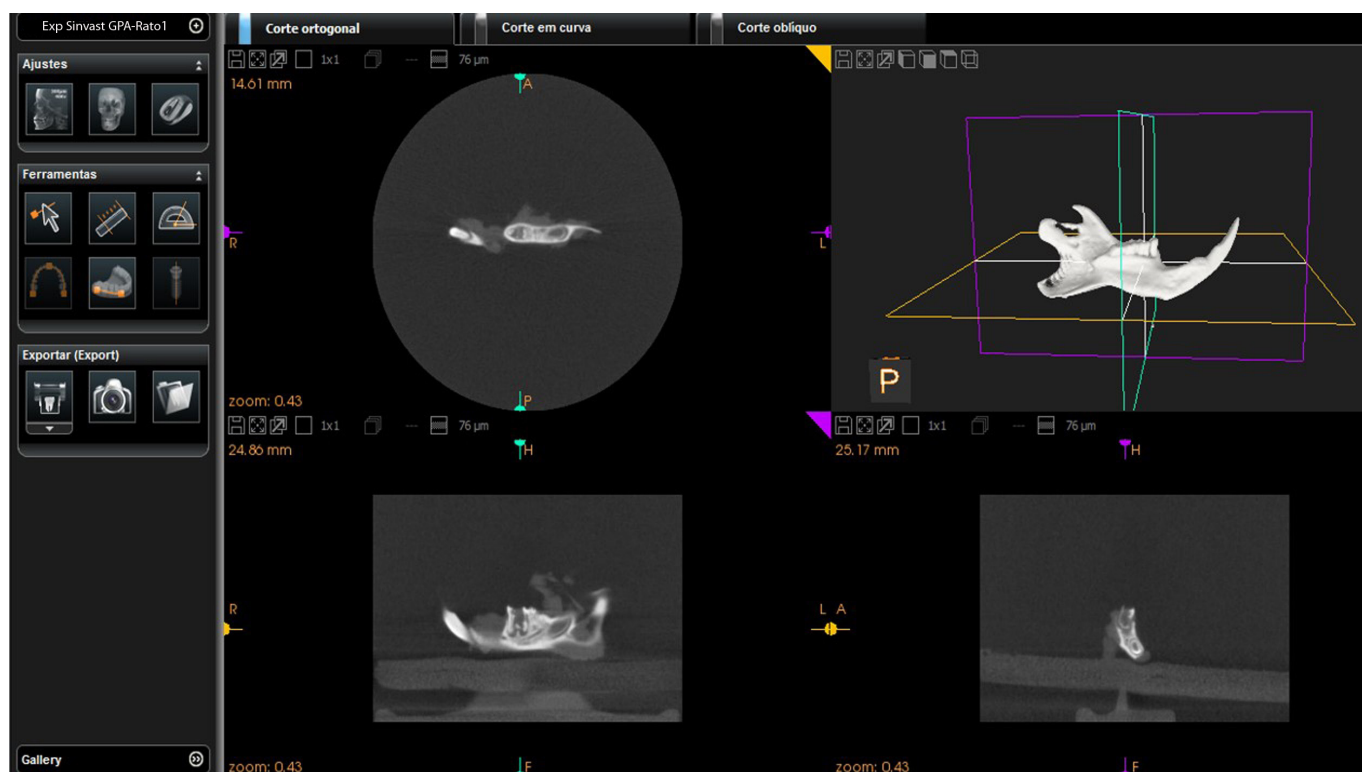


Figure 1. Demonstrative figure of the tomographic images selected or perimeter analysis of increased apical ligament spaces.

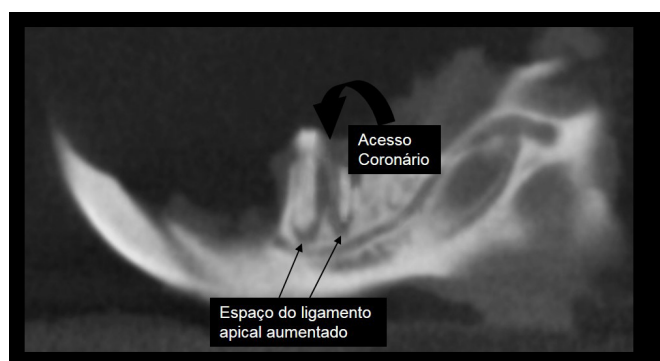


Figure 2. CT scan of the induced apical periodontitis associated with the simvastatin Group (APSG) and increased apical ligaments of the lower first molar.

The blood parameters were observed after measuring the quantities of glucose, HDL, VLDL, LDL and triglycerides.

For statistical analysis, the original data were subjected to preliminary testing to verify the normality of the sample (Shapiro-Wilk). Comparisons among the mean images, animal weights and biochemical data were performed using univariate analysis of variance (ANOVA) test with Tukey's post hoc (IBM SPSS Statistics Version 20).

To assess the calibrations of a single study investigator unaware of the study groups, we used Student's t test. The significance level for all tests was 5%, with a mean standard error of 0.035 mm.

RESULT

The perimeters of the periapical ligament spaces in the first molars were measured. The results demonstrated that APG had highest apical lesion progressions, followed by APSG and GC, respectively, with statistical differences observed between the groups ($p < 0.05$) (Table 1).

Table 1. Means and Standard Deviations of the periapical ligament perimeters

Groups	N	Means (mm)	Standard Deviations
CG	12	0.64A	243.55
APSG	12	1.54B	780.34
APG	12	3.42C	655.51

Capital letters A, B and C indicate statistical differences between the groups. CG – Control Group; APSG – Apical Periodontitis associated with Simvastatin; and APG – Apical Periodontitis Group. (One-way ANOVA $p < 0.05$).

Regarding blood biochemistry, the drug action was observed in several tests compared with the APG and CG. With respect to the glucose, triglycerides, HDL and VHDL variables, the medication demonstrated direct action compared with other groups ($p < 0.05$). However, the variables for cholesterol and LDL showed no significant differences between the groups ($p > 0.05$) (Table 2).

Regarding the weight differences of these animals, a significant difference was observed between the groups, with the following decreasing order: APG, CG and APSG ($p < 0.05$) (Table 3).

DISCUSSION

The aim of the present study was to obtain an understanding of the biological plausibility of the induced apical periodontitis progression associated with simvastatin drug use. The results demonstrated that this modulation offers an interesting initial tool for drug use in the treatment of periapical disease in rats.

Table 2. Means and Standard Deviations of the lipid components and glucose measured in blood tests for the animals in APSG, APG and CG

Variable	Groups	N	Mean mg/dL	Standard Deviations
Glucose	CG	12	152.46A	26.76
	APSG	10	110.79B	14.32
	APG	10	145.53A	19.74
Cholesterol	CG	12	98.80A	20.20
	APSG	10	86.04A	15.02
	APG	10	92.29A	18.10
Triglycerides	CG	12	98.90A	19.62
	APSG	10	75.50B	28.12
	APG	10	93.81A	23.14
HDL	CG	12	47.50A	4.57
	APSG	10	43.16A	5.42
	APG	10	30.97B	7.96
LDL	CG	12	31.52A	8.05
	APSG	10	27.73A	14.10
	APG	10	28.61A	11.97
VLDL	CG	12	19.78A	3.92
	APSG	10	13.39B	5.62
	APG	10	15.10B	3.49

Capital letters A and B indicate significant differences between the groups. APSG – Apical Periodontitis associated with the Simvastatin Group; APG – Apical Periodontitis Group; CG – Control group. (One-way ANOVA $p < 0.05$). HDL – high-density lipoprotein; LDL – low-density lipoprotein; VLDL – very low density lipoprotein.

Table 3. Means and Standard Deviations of the weight differences (in grams) between the final and initial measurements of the animals in APSG, APG and CG (N=12)

Groups	Mean of Initial Weight	Mean of Final Weight	Difference of Final and Initial Weight	Standard Deviations
GC	216.44A	287.56A	71.12A	12.51
GPAS	213.80A	277.02B	63.22B	10.96
GPA	218.22A	311.28C	93.06C	15.50

Capital letters A, B and C demonstrate significant differences between the groups. APSG – Apical Periodontitis associated with Simvastatin Group; APG – Apical Periodontitis Group; CG – Control group. (One-way ANOVA $p < 0.05$).

Currently, obesity affects much of the population¹⁷ and causes serious problems related to cardiovascular disease⁶, diabetes, chronic kidney disease, stroke, and other associated diseases¹⁸. Previous studies have suggested a relationship between inflammation and overweight or even morbid obesity in humans¹⁹. Indeed, fat cells, through the production of interleukins called adipokines and, more specifically, resistin, leptin and adiponectin, interfere with inflammatory processes associated with the use of simvastatin for the treatment of apical lesions²⁰. Because simvastatin plays a role in the reduction of inflammation, it is possible to decrease the amount of cholesterol and interleukins to prevent bone resorption.

Recent epidemiological data have indicated a weak relationship between obesity and dentistry²¹, but the plausibility of this hypothesis needs further evidence to clarify this issue^{22,23}. Notably, individuals who regularly permanently use simvastatin have shown a significant decrease in blood cholesterol levels and a consequent reduction of inflammation in the body and medical complications associated with obesity. Based on this finding, research concerning the use of simvastatin and evaluation of oral health indicators, as conducted in the present study, could provide important data for understanding the cause/effect relationship between pulp tissue aggression and apical lesion progression.^{20,21}

Alterations in the cardiovascular system have been associated with dyslipidemia worldwide²⁴. To treat these complications, simvastatin, a member of the statins group, has been widely used due to its low cost, easy acquisition and few side effects²⁵. Among its many demonstrated functions, simvastatin reduces the lipids in the bloodstream and has been associated with decreased and modulated inflammatory processes²⁵, apoptosis regulation, and changes in fibroblast function and bone formation¹¹. Despite not having original indications, simvastatin has recently been explored for its physical and chemical properties in dentistry^{26,27}. However, the pharmacokinetics of this drug should be considered to maximize the results, particularly concerning security in the use of this compound.

The systemic findings are relevant. The biochemical results presented in the present study showed clear systemic changes

in the blood volume of most of the indicators analyzed. No side effects were observed in the animals examined in the present study. We also considered the central nervous system behavior through labyrinth devices in high cross and open fields²⁸ and systemic indicators of drinking water, food and clinical symptoms, such as weight loss and diarrhea.

The hypothesis that simvastatin reduces the amount of inflammatory interleukins is based on the reduction of these inflammatory products and by-products in the bloodstream. Inflammatory interleukins provide the local reduction of inflammation, even in the face of an infectious process¹³. This feature is important because stimulation of the immune inflammatory system results in the avoidance of the action of the biofilm in the progression of infectious diseases^{29,30}. Thus, two studies have suggested the use of this drug in the treatment of diseases, such as apical periodontitis^{15,16}.

The pathogenesis of endodontic diseases remains unknown in many respects. However, in general, endodontic diseases result from biofilm formation by microorganisms such as *Candida albicans*, *Enterococcus faecalis*³⁰, and *Porphiromona endodontalis*³¹. The inclusion of simvastatin in this infectious-inflammatory process will provide additional information on the action of this drug in the periodontium, achieving a methodology for the use of this medication and proper dosage to provide periapical-induced disease modulation, as analyzed using an experimental rat model.

Other clinical and laboratory relevant issues include the simvastatin dose (six milligrams) used in the present study, as established in previous pilot studies and supported by previously published manuscripts^{9,16}.

In addition, the target of the lipid and glucose profile changed both in the pilot study period and during the experimental phase of the study. Among the present findings, inflammatory processes leading to significant systemic changes, such as changes in weight and blood, were observed, revealing that simvastatin acts in any inflammatory process, including the oral cavity.

Notably, between the collection and arrival of the blood in the laboratory, the hemolysis of two samples from APSG and two samples from APG was observed. While no animals died during the experiment, the sample size of the groups decreased, despite the sufficient sample strength.

Indeed, these results are interesting and provide plausible information for clinical studies to increase the current understanding of the dynamics underlying the action mechanisms of a drug already established in the market, showing low cost, reasonable accessibility and diminished side effects.

CONCLUSION

Based on these results, we concluded that simvastatin use results in smaller apical ligament spaces subjected to the induction of periapical disease in the teeth of rats.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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