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±0,65) apresentou os maiores perímetros para espaço do ligamento periapical, seguido pelo GPAS (1,54±0,78) e GC (0,64±0,24), respectivamente (p<0,05). Em relação ao lipidograma percebe-se o efeito da simvastatina avaliando-se a quantidade de glicose, triglicerídeos, HDL, VLDL (p<0,05). Para a massa corporal o GPA foi o que mais ganhou peso (264,75±44,11); seguido pelo GC (252,00±44,36); e GPAS (245,41±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 GC (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 worldwide. The most common oral diseases include tooth decay and periodontitis. The focus on infection-induced biofilm in the oral cavity is an important element in the inflammatory response generated by the body and results in other problems associated with the well-being and health of the person.

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 disease.

The systemic mechanism of action of SVT has recently been explored, 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 effects.

In dentistry, several studies have attempted to associate SVT with repair and bone regeneration, 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 diseases. Thus, the use of SVT as an adjunct in the treatment of marginal periodontitis has recently become a research field.

Studies concerning apical periodontitis induction are relevant 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 rats has been proposed as a plausible contribution to research information that cannot be used in human subjects.

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. 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, Abirbrands, 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 weight.

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 administrated 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, Abirbrands, 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 weight. 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.

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).
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).

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.
Currently, obesity affects much of the population and causes serious problems related to cardiovascular disease, diabetes, chronic kidney disease, stroke, and other associated diseases. Previous studies have suggested a relationship between inflammation and overweight or even morbid obesity in humans. 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 lesions. Because simvastatin plays a role in the reduction of inflammation, it is possible to decrease the amount of cholesterols and interleukins to prevent bone resorption.

Recent epidemiological data have indicated a weak relationship between obesity and dentistry, but the plausibility of this hypothesis needs further evidence to clarify this issue. 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.

Alterations in the cardiovascular system have been associated with dyslipidemia worldwide. 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 effects. Among its many demonstrated functions, simvastatin reduces the lipids in the bloodstream and has been associated with decreased and modulated inflammatory processes, apoptosis regulation, and changes in fibroblast function and bone formation. Despite not having original indications, simvastatin has recently been explored for its physical and chemical properties in dentistry. 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 fields 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 process. 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. Thus, two studies have suggested the use of this drug in the treatment of diseases, such as apical periodontitis.

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, and Porphyromonas endodontalis. 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.

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 AG 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.

ACKNOWLEDGEMENTS

The authors would like to thank the Master in Integrated Dental Sciences, University of Cuiabá - UNIC for supporting this manuscript and the Radiology Clinic CEDROC for providing the data for the present study.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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Received: October 2, 2015
Accepted: May 2, 2016