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The Role of Scaling-Root Planing with a Subgingival Application of Tetracycline 0,7 % Based Chitosan Hydrogel in Periodontitis Rats' Model (Evaluation of Clinical Parameters and Fibroblast Cell Counts on Periodontal Ligaments)

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Abstract

Introduction: Periodontal disease is a disease caused by bacterial plaque activity which cause a destruction to the periodontium. The purpose of scaling and root planing (SRP) is to eliminate bacteria plaque, calculus and to create a smooth root surface. Tetracycline 0,7% based chitosan hydrogel was a local delivery antibiotic (LDA)that consist of a broad-spectrum antibiotic and an anti-collagenase property which help in regeneration of the connective tissue by increasing the secretion of the fibroblast

Purpose: The purpose of this study is to evaluate the changes of the clinical parameters and the amount of fibroblast cells through histological evaluation

Material and Methods: The total of 27 male *Wistar* rats were used in this study and divided into three group. The interventionofthefirstgroupwas SRP with a sub gingival applicationofLDAfor 7 days. The intervention for the second group was SRP with a sub gingival application of LDA only once. The intervention for the third group was only SRP. The clinical parameter was evaluated at the baseline and the 14 days together with the histological evaluation.

Results: This study shows that the group with a sub gingival application of tetracycline 0,7% based chitosan hydrogel for 7 days had the greatest change on clinical parameters and best secretion of the fibroblast cells also it was statistically significance (*p<0,05*).

Conclusion:SRP with asubgingival application of tetracycline 0,7% based chitosan hydrogelfor 7 days gave the best result on the clinical parameters changes and a better amount of fibroblast cells secretion.

Keywords: Periodontal Disease, Tetracycline Based Chitosan Hydrogel, Fibroblast Cell

1. Introduction

Periodontal disease is a disease caused by bacteria that affect the supporting tissues of the teeth. Periodontitis is one of the main causes of tooth loss and considered to be one of the biggest threats to oral health and this disease is often called as the silent disease.[1] In the human oral cavity, there are approximately ± 800 species of bacteria that can be found, bacteria species such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Prevotella intermedia, Campylobacter rectus dan Eikenella corrodens.* The complex interaction of these bacteria and the host response often be the main cause of periodontal disease. [1,2]

Mechanical debridement of dental plaque and elimination of local irritant factors are the basic of initial periodontal therapy.[1] This procedure includes scaling and root planing (SRP), dental health education (DHE) and periodic visits for oral hygiene care. The aim of SRP is to eliminate dental plaque, calculus and to create a smooth root surface which is a major factor in the occurrence of periodontal disease. The effectiveness of the treatment is seen through the reduction of clinical symptoms and the bacteria pathogen of periodontal disease.[1,3]

In the cases of deep periodontal pockets, treatment that consist of SRP only is not enough. Therefore, an adjuvant therapy is needed such as the use of antibiotic which help to reduce or stop the development of bacteria in the periodontium and also to reduce the severity of gingival inflammation which can be examined using the clinical parameters such as the modified papillae bleeding index.[4,5]

Antibiotics can be given systemically or locally, systemic administration gives more favorable results because the antibiotics can penetrate into the periodontium and periodontal pockets through the serum and reaches the microorganisms contained in the pocket.[1] However, systemic administration can disrupt the normal flora in the oral cavity when given in a long period of time.[4] Therefore, subgingival local administration has more benefits where it can penetrate into deep pockets with aminimal dose, directly contact with the pathogenic bacteria and also to minimize the side effects of the drugs.[6]

Tetracycline is the most common local antibiotic that is used. Tetracycline has an anti-collagenase effect which help in clinical attachment gains and regeneration of the tissue through promoting the attachment of fibroblast cells and reducing the destruction of the periodontium tissue and bone loss.[5,7]

Chitosan is polycationic with PH <6 that can interact with negative molecular ions such as protein & anionic polysaccharides.[8]Chitosan has good biodegradability, non-toxicity, anti-bacterial effect and good biocompatibility. Chitosan has an advantage in wound healing because it can stimulate homeostasis and accelerate tissue regeneration.[8,9]

Fibroblasts are predominant cells in the periodontium tissue. The main function of fibroblast is toproduce fibers and active intracellular substances.[10] Fibroblast also play a role in forming and placing fibers in the matrix, especially collagen which is abundant found in the periodontal ligament area.[11]

The purpose of this study was to compare the effectiveness combination therapy of scaling and root planning with the subgingival application of tetracycline 0,7% based chitosan hydrogel as a local delivery antibiotic (LDA) along toward the group without application of local delivery drug as a treatment of chronic periodontitis in rats' model.

2. Materials and Method

This study was a laboratory experimental research with a pre and post-test control group design on the clinical parameter and post-test only control group design on the histologic parameter.The ethical clearance for undertaking this study were obtained from the Health Research Ethics Committee faculty of mathematics and natural science of Universitas Sumatera Utara for animal conduct study and also from the Health Research Ethics Committee faculty of medicine of Universitas Sumatera Utara for the histologic study conduct. The animals were maintained in accordance with Committee for the Purpose of Control and Supervision of Experimental Animals guidelines for the care and use of laboratory animals.

2.1 Preparation of Tetracycline 0,7% Based Chitosan Hydrogel

The method of manufacturing the tetracycline 0,7 % based chitosan hydrogelwas based on the research reported by Susanto Cet al in 2016.12 The process starts by weighting the chitosan, tetracycline powder and lactic acid 1% respectively.Firstly, the chitosan was added into the stamper. Secondly, the 1% of lactic acid was added into the stamper and stirred until a homogenous chitosan hydrogel was created. Lastly, the tetracycline which has been crushed was added and stirred until a homogenous tetracycline 0,7 % based chitosan hydrogel was created. After that, the gel was transferred to 1cc syringe and kept in the cold storage with a temperature of 4oC.This procedure was done aseptically in the laminar air flow and all equipment were sterilized in autoclave before used.

2.2 Creating Periodontitis Rats' Model

The experiment begins with the procedure of creating a periodontitis rats' model with a total of twenty-seven (27) healthy Wistar rats with a weight of 200-250 gram. The procedure begins with an intraperitoneal anesthesia consists of ketamine and xylazine to produce a sedation effect on the rats. Sulcular incision with a full thickness flap were raised in the vestibular area of both lower central incisors. The bone defect was created by using the carbide bur with the help of low speed micromotor then the flap was repositioned to the original positioned and horizontal mattress suture with a 5-0 silk was done. The rats were given a high carbohydrate diet to ensure an accumulation of plaque. One week later, the suspension consists of P. gingivalisbacteria was injected in the sulcus area and a swab was performed three days after the injection to ensure the presented of P. gingivalisbacteria.

2.3 The Treatment and Control Group in Periodontitis Rats' Model

The total of twenty-seven (27) periodontitis rats' model were divided into three (3) group: Group (A) with a treatment of SRP and asubgingival application oftetracycline 0,7 % based chitosan hydrogelevery day for seven (7) days, group (B) with a treatment of SRP andasubgingival application of

tetracycline 0,7 % based chitosan hydrogelonly on the first day and group (C) with a treatment of SRP only as a control group. The clinical parameter of papillary bleeding index (PBI) wasrecorded at the baseline before the treatment procedure and fourteen days post treatment respectively in each group. The rats were kept in clean, hygienic cages and maintained under standard laboratory conditions. The rats were kept in groups of four per cage at controlled temperature (25°C ± 2°C) with 12 h light/dark cycle and humidity. They were given the standard diet and water adlibitum.

2.4 Preparation of Histologic Study Specimen

The rats were euthanasia on day 14 using ether inhaled, then the rat's mandible was taken and put in pots containing 10% formalin buffer. The preparation of microscopic specimen begins by obtaining the tissue sample and put it into the diskette then thefixation of the specimen was done using an automatic tissue processor. After that, the samples were infiltrated with a liquid paraffin and put in a freezer for a while then each paraffin block is sliced into 3-4 µm thick tissue using a microtome. The slices tissue was put into the water bath and rehydrated then the staining ofHarris Hematoxillin-eosin was performed. The fibroblast cells count was seen and count on five fields of view using a microscope with 400 times magnification and the help of image processing software programs.

2.5 Data Analysis

The data obtained in this study were quantitative data in the form of clinical parameters before and after treatment as well as histological examination of the number of fibroblasts cells count with a ratio scale.The normality test was analyzed using the Shapiro-Wilk test to determine the normality of the data. The Anova test was performed on the normal distributed group. Data that considered deviated from the normal distribution were analyzed using Wilcoxon and Kruskal-Wallis test.

3. Results

The Clinical Parameter Data Analysis

The clinical parameter data analysis of papillary bleeding index (PBI) result of the three different group will be shown from table 1 to table 2 below.

Table 1. Difference of papillary bleeding index mean value on baseline and 14 days post treatment in each group

Group	**PBI Mean Value ($\bar{x}$ ± SD)**		**P value**
	Baseline	**14 days Post treatment**	
SRP + LDA for 7 days	2.22± 0.79	0.33± 0.36	0.007*
SRP + LDA only on 1st day	2.61 ± 0.41	0.94± 0.39	0.007*
SRP	2.55 ± 0.39	1.72± 0.44	0.027*

Wilcoxon test, PBI=papillary bleeding index, SRP=scaling and root planing, LDA= local delivery antibiotic,significant P<0.05,

According to the table 1 above, it shows that there was significant decrease of PBI in all group which is statistically significant (P< 0.05).

Table 2. Comparison test to determine the papillary bleeding index changes 14 days post treatment in each group

Group	**PBI changes ($\bar{x}$ ± SD)**	**P value**
SRP + LDA for 7 days	1.89 ± 0.78	0.008*
SRP + LDA only on 1st day	1.67 ± 0.50	
SRP	0.83 ± 0.75	

Anova test, PBI=papillary bleeding index, SRP=scaling and root planing, LDA= local delivery antibiotic,significant P<0.05,

According to the table 2 above, it shows that the greatest change of PBI after 14 days was seen on the group with a 7 days treatment of LDA and statistically significant (P< 0.05).

3.1 The Histologic Parameter Data Analysis

The histologic parameter data analysis of fibroblast cell count in the ligament periodontal area of the three group will be shown on table 3 below.

Table3. The differences of total fibroblasts cell count 14 days post treatment in each group

Group	**Total of Fibroblast Cell Count ($\bar{x} \pm$ SD)**	**P value**
SRP + LDA for 7 days	48.24 ± 9.19	<0.001*
SRP + LDA only on 1st day	33.40 ± 5.28	
SRP	31.24 ± 6.32	

Anova test, SRP=scaling and root planing, LDA= local delivery antibiotic, significant P<0.05,

According to the table 3 above, it shows that the number of fibroblast cells was greater in group with 7 days LDA treatment compared to the others group and it is statistically significant (P<0.05).

Figure 1

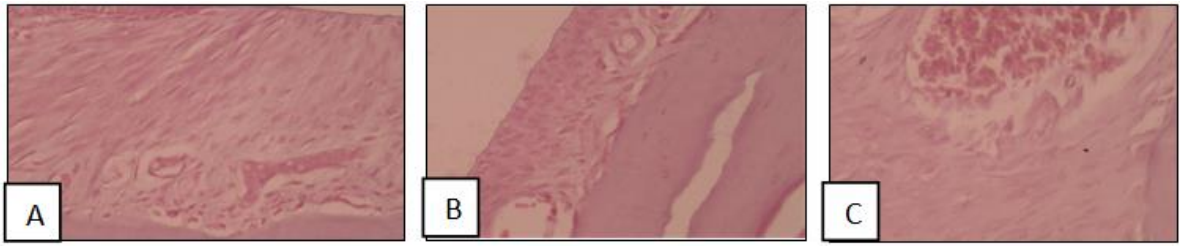

Figure 1. Histological view of the number of fibroblast cells in the periodontal ligament with a Hematoxylin Eosin (H&E) staining in one sample from each group. A. Histological view of the number of fibroblast cells in the group with combination of SRP and 7 days LDA treatment. B. Histological view of the number of fibroblast cells in the group with combination of SRP and LDA treatment only on the 1st day. C. Histological view of the number of fibroblast cells in the control group.

According from the figure 1, it shows that no inflammatory cells was found and the number of fibroblast cells count also greater in the group with combination treatment of SRP and 7 days application of LDA.

4. Discussion

The results of this study indicate the papillary bleeding index (PBI) score appear to be better and statistically significant (p <0.05) in the group with a combination therapy of scaling and root planing with a subgingival application of 0,7% tetracycline-based chitosan hydrogel for 7 days compared to the other groups.

Scaling and root planing (SRP) is an inseparable part in the treatment of periodontal disease where the aim of SRP is to eliminate biofilms containing bacterial pathogens and calculus attached to the tooth surface both in supragingival and subgingival areas and to create a smooth root surface which helps in the attachment to the gingiva.[13] Nitesh, et al stated that mechanical debridement alone cannot

completely eliminate bacteria such as A. actinomycetemcomitans and P.gingivalis because these bacteria can invade and destroy the subepithelial connective tissue area. Therefore, the application of local antibiotic therapy provides an advantage because it can penetrate into the pocket area and reduce an inflammation in the tissue area .[14]

A study conducted by Grover HS, et al with a three-group treatment which is group of SRP with an application of fiber tetracycline, group of SRP with an application of chlorhexidine and control group on 48 subjects.[15] The study shows that there were no difference significant changes of PBI score in all group at the early time of the study. But the control on the 1st and 3rd month shows a greater change of those parameter on group with an application of tetracycline fiber. Another study conducted by Reddy S, et alwith a same method and same number of subject but with a different observation time shows that the group of SRP with an application of tetracycline fiber had a greater change in PBI score after 1 year.[16]

Akincibay H, et al on his clinical human study with a three-group treatment which is group of SRP with an application of chitosan, group of SRP with an application of local antibiotic-based chitosan and control group on 15 subjects, reported that a group with a combination of SRP therapy with an application of local antibiotic-based chitosan give a better change in PBI score and no clinical sign of inflammation with a stable periodontal health was found.[17] Another study conducted by Babrawala IS, et al on 10 subjects comparing between a group of SRP therapy with an application of local antibiotic-based chitosan and SRP alone shows the similar results with the previous study.[18]

In the present study it also indicates that the number of fibroblast cells count in the periodontal ligament area is better in the group with the combination therapy of SRP with a subgingival application of 0,7% tetracycline-based chitosan hydrogel for 7 days compared to the others group. Study conducted by Pang, et al on 30 males *wistar*rats shows that chitosan induced the proliferation and secretion of fibroblast cell and help in the production of type I collagen fibers and facilitate the forming of osteogenic cell which accelerated the healing of tissue and bone.[19] A study conducted by Pradyani IGAS, et al about the effectiveness of SRP with an application of tetracycline 0,7% gel on 32 male rats shows that there was a significant increase of fibroblast cell in the periodontal ligament area compare to the control group.[20]

Hamilton V, et al on his study about chitosan characteristic shows that chitosan was very biocompatible to human fibroblast cell and it has a very good attachment of toward the fibroblast cells.[21]Arancibia, et al reported that chitosan helps in the inhibition of PGE_2 which helps in the reducing the degree of inflammation and increasing the secretion of fibroblast cells.[22] A study conducted by Lima AC, et al about the effect of SRP combine with an application of tetracycline with an observation on 24,48 and 72 hours on 40 samples. It shows that the combination therapy gave a better adhesion of fibroblast cells and a better construction of fibrin network resulting in a better formation of collagen fiber.[23]

In the present study, we reported that a combination therapy of scaling and root planing with a subgingival application of 0,7% tetracycline-based chitosan hydrogel for 7 days was proven to

significantly decrease the papillae bleeding index and had a greater number of fibroblast secretions. Tetracycline is a broad-spectrum antibiotic with an anti-collagenase property that provide advantages over other antibiotics that affect stimulation, proliferation and increase the adhesion of fibroblast cells. Chitosan has a many great property advantages such asanti-bacterial and homeostasis effect which help in wound healing. The combination of these two materials give a very good advantage which result in a faster and betterhealing of periodontal tissue area.

5. Conclusion

In this study, we observe that combination therapy of scaling and root planing with a subgingival application of 0,7% tetracycline-based chitosan hydrogel for 7 days give a better result in the decreasing the papillary bleeding index and also a better secretion of fibroblast cells in the periodontal ligament area which result in a better healing of periodontal tissue area.

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