QUADRANT SCIENTIFIC PUBLISHERS

Journal of Clinical Trails & Research
Volume 1, Issue 1 - 2023, Pg. No. 20-23
Open Access/Peer Reviewed Journal

Research Article

An *In Vitro* Analysis Of Natural And Allopathic Antimicrobial Agents Onto Guided Tissue Regeneration Against Periodontal Pathogens

Parth Mehra[1*], Amrita[2]

[1,2]Post Graduate Student, Department of Periodontology and Implantology, Institute of Dental Sciences, India

Recieved: July 26, 2023
Accepted: July 27, 2023
Published: Aug 14, 2023

How to cite this article: Parth Mehra[1*], Amrita, Post Graduate Student, Department of Periodontology and Implantology, Institute of Dental Sciences, India. Journal of Clinical Trails & Research, 2023; 1(1): 20-23.

Corresponding Author: Parth Mehra, Post Graduate Student, Department of Periodontology and Implantology, Institute of Dental Sci Sciences, India.

Email:
parthmehra95.pm@gmail.com

ABSTRACT

Background
It is well established that plaque control is a critical determinant of the success or failure of various outcomes of periodontal therapy.

Materials and Methods
Tetracyclines in their purest form were obtained for the investigation. Pure green coffee extract, which contains 50% Chlorogenic Acid (CGA), was purchased from Top Secret Nutrition Products. Following microbial analysis, the producer confirmed that it was free of all types of bacteria, yeast, and mould. For each bacterium, the microbiological process was carried out three times. GTR membrane treated with distilled water served as the research's control. SPSS 21.0 software was utilized for the study's statistical analysis. The Kruskal Wallis ANOVA and Mann-Whitney U test were used to collect and analyze the statistical data.

Results
Zones of inhibition of *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)* and *Porphyromonas gingivalis (P. gingivalis)* by 1% tetracycline, 1% metronidazole, 1% green coffee extract, and distilled water. With regard to both bacterial species, distilled water and green coffee extract displayed no zone of inhibition. While 1% tetracycline showed the broadest zone of inhibition across both *A. actinomycetemcomitans and P. gingivalis*. The mean zone of inhibition for each bacterium for each antimicrobial agent used in the research was determined for assessment.

Conclusion
The number of periodontal bacteria colonizing the GTR membrane can be efficiently reduced by using readily available antimicrobial medications in such a low concentration.

Keywords: Tetracyclines, GTR membrane, Zones of inhibition

INTRODUCTION

It is well established that plaque control is a critical determinant of the success or failure of various outcomes of periodontal therapy.[1,2] Data indicate that decreasing the biofilm load may lead to improved clinical results and more predictable periodontal healing.[3,4] Placing bone grafts and membranes in infected sites may limit periodontal healing success.[5]

It was discovered that both non-biodegradable and biodegradable membranes might act as a nidus for the development of the microorganisms kept in the mouth cavity throughout the surgical and postoperative healing stages.[6] Additionally, the clinical features of the surgical site may at first aid in bacterial contamination and afterward cause extra healing difficulties.[7] Multiple strategies for the use of antimicrobial agents in regenerative procedures delivered by rinsing, irrigation, systemic administration, and local delivery systems have been addressed in multiple investigations due to the infectious nature of periodontal disease and the potential for contamination of regenerative substances.[8,9]

Through the use of physical obstacles for tissue separation and the promotion of the phenotypic expression of tissues, the fundamental idea behind GTR therapy [10] has opened the door to the pursuit of periodontal regeneration. The regeneration ability of cementum and alveolar bone,

especially in the context of considerable new connective tissue attachment creation following traditional GTR therapy, has been consistently shown to be typically constrained. After GTR therapy, partial regeneration is frequently observed in this area. A regenerative membrane should have a variety of capabilities in addition to serving as a physical barrier for tissue segregation to promote desired regeneration. Hard- wick et al.[11] indicated that the goal of regenerative membrane techniques is to create a suitable environment in which the natural biological potential for functional regeneration can be maximized.

Tetracyclines (TCs) have been endorsed as beneficial supplements to periodontal therapy. The anti-collagenolytic, anti-inflammatory, osteoclast-inhibitory, and fibroblast-stimulatory qualities of these substances, along with their recently recognized non-antimicrobial activities, may contribute to their demonstrated efficacy. Clinical efficacy has been shown in the use of TCs in LDS for periodontal treatment. Furthermore, numerous investigations have demonstrated that topically putting TCs on experimental bone defects encouraged osteogenesis.

Tetracyclines and the GTR membrane may work well together to reduce barrier-associated infections during GTR therapy. The purpose of this in vitro experiment was to see if antimicrobial drugs applied to the surface of the GTR membrane could inhibit the growth of *A. actinomycetemcomitans and P. gingivalis.*

METHODOLOGY

The Institute of Dental Sciences in Bareilly's Department of Periodontology and Implantology served as the study's location. Tetracyclines in their purest form were obtained for the investigation. Pure green coffee extract, which contains 50% Chlorogenic Acid (CGA), was purchased from Top Secret Nutrition Supplements. After microbial analysis, the producer confirmed that it was free of all types of bacteria, yeast, and mould. One gram of these antimicrobial substances was dissolved in ten milliliters of distilled water to produce a 10% concentration of antimicrobial agent. Additionally, a sufficient quantity of solvent was used to dilute the concentrations to 1% for each of the three drugs (tetracyclines, metronidazole, and green coffee extract). A microbial test of the extracts was conducted. In this investigation, the solvent—distilled water—was utilized as a negative control.

The GTR membrane that was employed in the investigation was a Type-I collagen membrane that had been sterilely reconstituted. The membrane was sliced into 1x0.5 cm pieces using aseptic techniques. Nearly 10 ml of each of the following solutions were added to sterile Petri dishes before the GTR membrane was added: 1% tetracyclines, 1% metronidazole, and 1% green coffee extract solution. After spending 15 minutes soaking in the concentrated solution, the GTR membrane was dried for 5 minutes.

The antibacterial activity of the GTR membrane treated with 1% tetracycline solution, 1% metronidazole solution, and 1% green coffee extract solution, respectively, was assessed in vitro using the agar disc diffusion method. Different bacteria were cultured and examined on blood agar. Through the use of an inoculation loop, colonies of microorganisms were transported to the agar plate. The plates were rotated between each streak at around 60 degrees to ensure uniform dispersion of bacteria. Equivalent general inoculation and culture media inoculation procedures were used for *A. actinomycetemcomitans and P. gingivalis.*

On three distinct agar plates containing 1% tetracycline solution, 1% metronidazole solution, and 1% green coffee extract solution, respectively, the two bacteria were inoculated. Consequently, a total of six plates were injected with each of the microorganisms. Before putting the GTR membrane that had been treated with various antimicrobial compounds to be evaluated on the inoculation plates, they were allowed to stand for at least three minutes but no more than 15 minutes. The GTR membrane was applied, and the plates were incubated at 37 °C for 15 minutes.

A 48-hour incubation period was used for both anaerobic *(P. gingivalis) and aerobic (A. actinomycetemcomitans)* microorganisms. For the 48-hour cultivation of aerobic microorganisms, a 37°C incubator was utilized instead of McIntosh and Filde's anaerobic jar. Following the incubation time, the plates were read to see if the lawn of growth was confluent. Employing a Vernier caliper, the diameter of the zone of inhibition was measured to the closest millimeter. For each bacterium, the microbiological process was carried out three times. GTR membrane treated with distilled water served as the research's control.

SPSS 21.0 software was utilized for the study's statistical evaluation. The Kruskal Wallis ANOVA and Mann-Whitney U test were used to collect and analyze the statistical data. To assess the antibacterial property amongst several groups, the Mann-Whitney U test was used. The threshold for statistical significance was set at 0.05.

RESULTS

Zones of inhibition of *A. actinomycetemcomitans and P. gingivalis* by 1% tetracycline, 1% metronidazole, 1% green coffee extract, and distilled water. With regard to both bacterial species, distilled water and green coffee extract displayed no zone of inhibition. While 1% tetracycline showed the broadest zone of inhibition against both *A. actinomycetemcomitans and P. gingivalis*. The mean zone of inhibition for each bacterium for each antimicrobial agent used in the research was determined for analysis. Tetracycline showed level of significance against *A. actinomycetemcomitans* and *P. gingivalis* in contrast to other antimicrobial drugs and the research's negative control. Whereas, neither metronidazole nor green coffee extract showed any level of significance against *A. actinomycetemcomitans* using Mann-Whitney U test.

Table 1. Zones of inhibition for various anti-microbial agents.

Antimicrobial Extracts	Microorganisms Tested					
	A. actinomycetemcomitans			*P. gingivalis*		
Tetracycline	42 x 46	43 x 48	41 x 45	22 x 18	30 x 24	24 x 18
Metronidazole	16 x 16	0 x 0	10 x 10	18 x 14	18 x 16	17 x 14
Green coffee extract	0 x 0	0 x 0	0 x 0	0 x 0	0 x 0	0 x 0
Distilled Water	0 x 0	0 x 0	0 x 0	0 x 0	0 x 0	0 x 0

Table 2. Pairwise comparisons of four anti-microbial agents with a zone of anti-microbial activity of *A. actinomycetemcomitans* by Mann-Whitney U test.

Anti-Microbial Agents	Mean	SD	SE	Sum of Ranks	U-Value	Z-Value	p-value
Tetracycline	1947.00	110.27	63.66	15.00	0.00	-1.964	0.0495*
Metronidazole	118.67	129.02	74.49	6.00			
Amoxicillin	1947.00	110.27	63.66	15.00	0.00	-1.964	0.0495*
Green Coffee	0.00	0.00	0.00	6.00			
Tetracycline	1947.00	110.27	63.66	15.00	0.00	-1.964	0.0495*
Distilled Water	0.00	0.00	0.00	6.00			
Metronidazole	118.67	129.02	74.49	13.50	1.50	-1.3093	0.1904
Green Coffee	0.00	0.00	0.00	7.50			
Metronidazole	118.67	129.02	74.49	13.50	1.50	-1.3093	0.1904
Distilled Water	0.00	0.00	0.00	7.50			
Green Coffee	0.00	0.00	0.00	10.50	4.50	0.0000	1.0000
Distilled Water	0.00	0.00	0.00	10.50			

Table 3. Pairwise comparisons of four anti-microbial agents with a zone of anti-microbial activity of *P. gingivalis* by Mann-Whitney U test

Anti-Microbial Agents	Mean	SD	SE	Sum of Ranks	U-Value	Z-Value	p-value
Tetracycline	516.00	177.58	102.53	15.00	0.00	-1.964	0.0495*
Metronidazole	259.33	25.79	14.89	6.00			
Tetracycline	516.00	177.58	102.53	15.00	0.00	-1.964	0.0495*
Green Coffee	0.00	0.00	0.00	6.00			
Amoxicillin	516.00	177.58	102.53	15.00	0.00	-1.964	0.0495*
Distilled Water	0.00	0.00	0.00	6.00			
Metronidazole	259.33	25.79	14.89	15.00	0.00	-1.964	0.0495*
Green Coffee	0.00	0.00	0.00	6.00			

DISCUSSION

The GTR membrane's microbial colonization has a negative impact on the effectiveness of periodontal therapy.[12] Periodontitis and the loss of alveolar bone may occur more quickly if the periodontal bacteria attach to the GTR membrane. Direct antimicrobial chemical insertion into the GTR membrane might provide effective infection control. Tetracycline and amoxicillin loaded on the GTR membrane have been proven in prior experiments to significantly lessen the adhesion of *Streptococcus mutans* and *A. actinomycetemcomitans*.[13]

In the current investigation, Type-I collagen GTR membranes were loaded with a small amount of antimicrobial drugs to test their effectiveness a gainst *A. actinomycetemcomitans* and *P. gingivalis*. Enhanced periodontal therapy outcomes may result from the loading of the GTR membrane with commercially available antimicrobial medications, which can help in the eradication of locally-dwelling harmful microorganisms. The clinical ramifications of using such a negligible quantity of antibacterial medicines on the GTR membrane may improve. The goal of the current investigation was to determine whether different allopathic and herbal antimicrobial drugs had any effect on periodontogenic infections at a concentration as low as 1%.

The green coffee extract was placed onto the GTR membrane in this unique study to test its antibacterial effects on *A. actinomycetemcomitans* and *P. gingivalis*. Findings that are comparable to those of the current investigation were found by Hung S-L et al., who showed that *A. actinomycetemcomitans* and S. mutans had less adhesion to GTR membranes loaded with tetracycline and amoxicillin.13 The class of antibiotics known as nitroimidazoles includes metronidazole. Today, it is used to treat anaerobic and protozoal infections, though it was initially developed to treat Trichomonas vaginalis infections. The poisonous metabolites that metronidazole produces cause the bacterial cell's DNA to be broken, which is how it achieves its bactericidal effect.14

In the current investigation, metronidazole performed better against *P. gingivalis* than *A. actinomycetemcomitans* when coated over the GTR membrane. A comparable study found that when the GTR membrane was surface coated with metronidazole, clinical locations in patients showed a significant improvement. According to Dowell P et al., it also assisted in lessening the patient's post-operative discomfort.15 After topical administration of sustained-release antibiotics as well as subgingival irrigation with metronidazole, other writers observed comparable effects.[16,17]

CONCLUSION

The GTR membrane is one of the successful ways for periodontal regeneration, according to numerous studies, however, it is vulnerable to bacterial contamination, which hinders periodontal regeneration. Antibiotics used systemically may result in adverse effects and the development of drug resistance. As a result, applying antimicrobial medicines to the GTR membrane may stop bacteria from colonizing it. Tetracycline at a concentration of 1% in the current investigation demonstrated a substantial antibacterial activity against both aerobic and anaerobic micro-organisms. According to the results of the current investigation, using commercially available antimicrobial drugs in such a little concentration can successfully lower the number of periodontal pathogens colonizing the GTR membrane

REFERENCES

1. Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease. J. Clin. Periodontol., 1981;8:281-294.
2. Lindhe J, Westfelt E, Nyman S, Socransky SS, Haffajee AD. Long-term effect of surgical/non-surgical treatment of periodontal disease. J. Clin. Periodontol., 1984;11:448-458.
3. Sander L, Frandsen EV, Arnbjerg D, Warrer K, Karring T. Effect of local metronidazole application on periodontal healing following guided tissue regeneration. Clinical findings. J. Periodontol., 1994;65:914-920.
4. Yoshinari N, Tohya T, Kawase H, et al. Effect of repeated local minocycline administration on periodontal healing following guided tissue regeneration. J. Periodontol., 2001;72:284-295.
5. Nowzari H, MacDonald ES, Flynn J, London RM, Morrison JL, Slots J. The dynamics of microbial colonization of barrier membranes for guided tissue regeneration. J. Periodontol., 1996;67:694-702. 6.
6. Nowzari H, Matian F, Slots J. Periodontal pathogens on polytetrafluoroethylene membrane for guided tissue regeneration inhibit healing. J. Clin. Periodontol., 1995;22:469-474.
7. Mombelli A, Zappa U, Bra¨gger U, Lang NP. Systemic antimicrobial treatment and guided tissue regeneration. Clinical and microbiological effects in furcation defects. J. Clin. Periodontol., 1996;23:386-396.
8. Westling M, Tynelius-Bratthall G. Microbiological and clinical short term effects of repeated intracrevicular chlorhexidine rinsing. J. Periodontal. Res., 1984; 19:202-209.
9. Southard SR, Drisko CL, Killoy WJ, Cobb CM, Tira DE. The effect of 2% chlorhexidine digluconate irrigation on clinical parameters and the level of Bacteroides gingivalis in periodontal pockets. J. Periodontol., 1989; 60:302-309.
10. Melcher AH. Repair of wounds in the periodontium of the rat. Influence of periodontal ligament on osteogenesis. Arch. Oral Biol., 1970;15:1183-1204.
11. Hardwick R, Hayes BK, Flynn C. Devices for den- toalveolar regeneration: An up to date literature review. J. Periodontol., 1995;66:495-505.
12. Nowzari H, MacDonald ES, Flynn J, London RM, Morrison JL, Slots J. The dynamics of microbial colonization of barrier membranes for guided tissue regeneration. J. Periodontol., 1996;67:694-702.
13. Hung S-L, Ling Y-W, Wang Y-H, Chen Y-T, Su C-Y, Ling L-J. Permeability of Streptococcus mutans and Actinobacillus actinomycetemcomitans through guided tissue regeneration membranes and their effect on attachment of periodontal ligament cells. J. Periodontol., 2002;73:843-51.
14. Lamp KC, Freeman CD, Klutman NE, Lacy MK. Pharmacokinetics and pharmacodynamics of the nitoimidazole antimicrobials. Clin. Pharmacokinet., 1999;36:353-73.
15. Dowell P, Al-Arrayed F, Adam S, Moran J. A comparative clinical study: The use of human type I collagen with and without the addition of metronidazole in the GTR method of treatment of periodontal disease. J. Clin. Periodontol., 1995;22:543-49.
16. Pedrazzoli V, Kilian M, Karring T. Comparative clinical and microbiological effects of topical subgingival application of metronidazole 25% dental gel and scaling in the treatment of adult periodontitis. J. Clin. Periodontol., 1992;19:715-22.
17. Ainamo J, Lie T, Ellingsen BH, Hansen BF, Johansson LA, Karring T, et al. Clinical responses to subgingival application of a metronidazole 25% gel compared to the effect of subgingival scaling in adult periodontitis. J. Clin. Periodontol., 1992;19:723-29.