ORIGINAL ARTICLE

The Effect of Local Administration of Metronidazole from Carbonate Apatite-gelatin Film on the Healing of Chronic Periodontitis Post-Curettage

Ricky Wibowo[1], Sri Pramestri Lastianny[2], Retno Ardhani[3], Juni Handajani[4], Ahmad Syaify[2]

[1] Dental Private Practice, Lombok, Indonesia
[2] Department of Periodontology, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.
[3] Department of Dental Biomedical Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.
[4] Department of Oral Biology, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

ABSTRACT

Introduction: The criteria for a good dental material could penetrate optimally and have biocompatibility, chemical stability, and biodegradability. Carbonated apatite-gelatin film as a Drug Delivery System (DDS) is known to increase the effectiveness of metronidazole in the healing process of periodontal therapy. The aim of this study was to determine the effect of carbonated apatite-gelatin film adjunction as a metronidazole delivery system in cases chronic periodontitis of post-curettage using several clinical parameters. Evaluation is carried out through measurements of Relative Attachment Loss (RAL), Pocket Depth (PD), and Bleeding on Probing (BOP). **Materials and methods:** Subjects were from 45 chronic periodontitis patients who had periodontal pockets 3-5 mm depth at the Academic Dental Hospital UGM. Subjects were treated with initial scaling and root planing treatment as well as periodontal therapy in the form of curettage. Subjects were divided into 3 groups, namely CHA film, adjuvant metronidazole, and CHA-metronidazole film. Clinical evaluations were carried out on the 0th, 7th, 21st, and 28th days. Clinical parameters were evaluated RAL, PD, and BOP then analyzed using the Kruskal Wallis and Mann-Whitney tests. **Results:** Administering carbonated apatite-gelatin film as a delivery system for metronidazole in the healing of post-curettage chronic periodontitis obtained a higher reduction in RAL, PD, and BOP values compared to administering metronidazole itself. **Conclusion:** TAdministering carbonated apatite-gelatin film as a metronidazole delivery system has a higher effect of reducing RAL, PD, and BOP compared to metronidazole in healing chronic periodontitis post curettage.

Keywords: Carbonated apatite gelatin, Drug Delivery System, Metronidazole, Curettage, Periodontitis

Corresponding Author:
Ahmad Syaify, Dr., Sp.Perio(K)
Email: ahmad.syaify@ugm.ac.id
Tel : +62 816-672-420

INTRODUCTION

The stages of periodontal therapy consist of several stages, namely phase I (non-surgical phase), phase II (surgical phase), phase III (restoration phase), and phase IV (maintenance phase). Phase I periodontal treatments include scaling root planing and curettage (1). Periodontal treatment measures undertaken aim to eliminate the etiology of the disease and prevent recurrence. Curettage is the scraping of the gingival wall in periodontal pockets to remove inflamed soft tissue. Curettage works to eliminate local factors and reduce pocket depth by means of new attachments. Curettage has the disadvantages of limiting access to furcation, the distal area of the molar teeth, and concavity in the contours of the teeth (1).

The effectiveness of periodontal therapy can be improved by administering adjuvants both locally and systemically. Adjuvant therapeutic supplies include antibiotics such as metronidazole or other ingredients that can optimize healing. Giving adjuvant therapy locally has the advantage of higher tissue concentration and requires a shorter time (2). Metronidazole is known as a drug of choice in anaerobic gram-negative bacterial infection which is the main bacterium that causes periodontal disease. Topical application of metronidazole has a higher drug concentration in the target area and can minimize the side effects of systemic applications when compared to systemic administration of metronidazole. The administration of topical metronidazole as an adjunctive in periodontal therapy has the disadvantage of being low stability so it is easily dissolved in saliva (1, 3, 4).

Good material properties in the oral cavity have stable chemical properties, are able to deliver the compounds it carries, are biocompatible and allow optimal drug

penetration into tissues. Apatite gelatin carbonate is one material that has biocompatible, biodegradable properties and is capable of being used as a drug and protein delivery material. Gelatin is an organic material resulting from denaturation or degradation of collagen which has proven effective as a defect filler and wound healing, while apatite carbonate is a polymer formation that functions as a scaffold (5-7).

Adding gelatin to the apatite carbonate in the form of an apatite carbonate membrane increases strength, decreases porosity, and can play a role in the healing process. Modification of gelatin with synthetic or natural polymers such as apatite carbonate can optimize drug release in tissues or is called Drug Delivery System (DDS) which can increase proliferation and adhesion in the periodontal ligaments, facilitating oxygen transport and nutrient supply to adjacent tissues (2, 8, 9).

The innovation in this study is modification of gelatin with apatite carbonate as a Drug Delivery System (DDS) for metronidazole. It is expected that the scaffold on the gelatin carbonate apatite membrane will be filled with metronidazole and carried to the tissue without being dissolved by saliva or Gingival Crevicular Fluid (GCF) so as to optimize the therapeutic effect and release of metronidazole in the tissue (2, 8, 9). The objective of this study was to determine the effect of carbonated apatite-gelatin film adjunction as a metronidazole delivery system in cases chronic periodontitis of post-curettage using clinical parameters Pocket Depth (PD), Relative Attachment Loss (RAL), and Papillary Bleeding Index (PBI).

MATERIALS AND METHODS

A total of 45 periodontal pockets in chronic mild-moderate periodontitis patients who came to Periodontics Clinic of Academic Dental Hospital Universitas Gadjah Mada (RSGM Prof. Soedomo). Subjects were chosen with a randomized control trial method. Subjects consisted of male and female; patients aged 30-50 years old. The inclusion criteria were patients with periodontal pocket 3-5mm depth; willing to participate in the study by signing informed consent. The exclusion criteria were subjects who had systemic disease; smokers; subjects with antibiotics, anti-inflammatory; pregnancy. Sample determined by randomized control trial. Randomization was conducted by means of patients who have fulfilled the inclusion criteria being grouped randomly to be divided into 3 groups according to the treatment group. Researchers used the principle of blinding to divide groups.

The clinical parameters were relative attachment loss, pocket depth, and bleeding on probing. Relative attachment loss and pocket depth were measured on baseline, 21st, and 28th while bleeding on probing was measured on baseline, 7th, 21st, and 28th. The materials were apatite carbonate gelatin film, diagnostic kits, metronidazole solution, periodontal dressing, and ultrasonic scaler.

All patients within inclusion criteria were informed about the detail of the study and then signed the informed consent. Initial examination were general data recording, medical history, and extraoral and intraoral examination, followed by intial scaling root planning treatment. Seven days after initial treatment, the measurement of clinical parameters pocket depth, relative attachment loss, and bleeding on probing were carried out and defined as baseline data. Patients were then devided into 3 treatment groups of curretage with metronidazole only application, curretage with carbonate apatite gelatin film-metronidazole application, and curretage with carbonate apatite gelatin film only application. Clinical evaluation of clinical parameters pocket depth, relative attachment loss, and bleeding on probing were measured on 21th, and 28th for pocket depth and relative attachment loss while 7th, 21st, and 28th for bleeding on probing after treatment.

Ethical Clearance

The protocol of this study was approved by The Research Ethics Committee, Faculty of Dentistry, Universitas Gadjah Mada No. 0074/KKEP/FKG-UGM/EC/2019.

RESULTS

Table I shows that all three group take reduction at 21st and 28th on pocket depth and relative attachment loss and 7th, 21st, 28th on bleeding on probing. The highest pocket depth and relative attachment level reduction was on curretage + CHA film group at 21st with 2.57mm reduction on pocket depth and 2.5mm reduction on relative attachment loss. The highest pocket depth and relative attachment level reduction at 28th was curretage + metronidazol group with 0.83mm reduction on both pocket depth and relative attachment loss. The lowest score for bleeding on probing at 7th was curretage + CHA-metronidazole film with 0 score.

Table I: Mean difference with standart deviation on PD, RAL and median on BOP in all treatment group

		Curretage+ metronida-zole		Curretage+ CHA film-metroni-dazole		Curretage+ CHA film	
PD	Base-line	3.60	± 0.21	3.47	± 0.61	4.27	± 0.46
	21st	2.60	± 0.78	1.80	± 0.46	1.70	± 0.49
	28th	1.77	± 0.53	1.27	± 0.37	1.63	± 0.61
	21st-0th	1.00	± 0.73	1.67	± 0.65	2.57	± 0.53
	28th-0th	1.83	± 0.49	2.20	± 0.85	2.63	± 0.72

CONTINUE

Table I: Mean difference with standart deviation on PD, RAL and median on BOP in all treatment group (CONT.)

		Curretage+ metronida-zole	Curretage+ CHA film-metroni-dazole	Curretage+ CHA film
PD	28th-21st	0.83 ± 0.62	0.53 ± 0.55	0.07 ± 0.65
RAL	Base-line	12.90 ± 0.74	14.27 ± 0.70	12.33 ± 1.50
	21st	11.93 ± 0.78	12.53 ± 0.48	9.83 ± 1.80
	28th	11.10 ± 0.69	12.03 ± 0.61	9.57 ± 1.78
	21st-0th	0.97 ± 0.72	1.73 ± 0.70	2.50 ± 0.65
	28th-0th	1.80 ± 0.46	2.23 ± 0.80	2.77 ± 0.56
	28th-21st	0.83 ± 0.52	0.50 ± 0.53	0.27 ± 0.53
BOP	Base-line	2 (1-3)	2 (1-3)	2 (1-2)
	7th	1 (0-1)	0 (0-1)	0 (0-1)
	21st	0 (0-1)	0 (0-1)	0 (0-1)
	28th	0 (0-0)	0 (0-0)	0 (0-0)

Table II shows that comparison on all three group (curretage + metronidazole vs curretage + CHA film-metronidazole, curretage + metronidazole vs curretage + CHA film, curretage + CHA film-metronidazole vs curretage + CHA film) have significant difference at 21st-0th on pocket depth reduction followed with significant difference at 28th-0th and 28th-21st on curretage + metronidazole group compared with curretage + CHA film group on pocket depth reduction ($p<0.05$). Pocket depth reduction at 28th-0th and 28th-21st on curretage + metronidazole vs curretage + CHA film-metronidazole and curretage + CHA film-metronidazole vs curretage + CHA film don't have significant difference ($p>0.05$).

Table II: Pocket depth difference comparison in all treatment group (CONT.)

		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+-CHA film
28th-0th	Curretage+metronidazole	-	0.154	0.002*
	Curretage+CHA film-metronidazole		-	0.068
	Curretage+CHA film			-
		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+-CHA film
28th-21st	Curretage+metronidazole	-	0.177	0.005*
	Curretage+CHA film-metronidazole		-	0.086
	Curretage+CHA film			-

Table III shows that comparison on all three group (curretage + metronidazole vs curretage + CHA film-metronidazole, curretage + metronidazole vs curretage + CHA film, curretage + CHA film-metronidazole vs curretage + CHA film) have significant difference at 21st-0th on relative attachment loss reduction followed with significant difference at 28th-0th and 28th-21st on curretage + metronidazole group compared with curretage + CHA film group on relative attachment loss reduction ($p<0.05$). Relative attachment loss reduction at 28th-0th and 28th-21st on curretage + metronidazole vs curretage + CHA film-metronidazole and curretage + CHA film-metronidazole vs curretage + CHA film don't have significant difference ($p>0.05$).

Table III: Relative attachment loss difference comparison in alltreatment group

		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+CHA film
21st-0th	Curretage+metronidazole	-	0.002*	0.000*

CONTINUE

Table III: Relative attachment loss difference comparison in alltreatment group (CONT.)

		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+CHA film
21st-0th	Curretage+CHA film-metronidazole		-	0.006*
	Curretage+CHA film			-
		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+CHA film
28th-0th	Curretage+metronidazole	-	0.107	0.000*
	Curretage+CHA film-metronidazole		-	0.051
	Curretage+CHA film			-
		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+CHA film
28th-21st	Curretage+metronidazole	-	0.085	0.009*
	Curretage+CHA film-metronidazole		-	0.344
	Curretage+CHA film			-

Table IV shows that there is significant difference between curretage + metronidazole vs curretage + CHA film-metronidazole and curretage + metronidazole vs curretage + CHA film at 7th on bleeding on probing score. There is no significant difference between curretage + CHA film-metronidazole vs curretage + CHA film at 7th on bleeding on probing score.

Table IV: Bleeding on probing difference comparison in all treatment group

		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+-CHA film
7th	Curretage+metronidazole	-	0.011*	0.001*
	Curretage+CHA film-metronidazole		-	0.291
	Curretage+CHA film			-

CONTINUE

Table IV: Bleeding on probing difference comparison in all treatment group (CONT.)

		Curretage+metronidazole	Curretage+CHA film-metronidazole	Curretage+-CHA film
7th	Curretage+CHA film-metronidazole		-	0.291
	Curretage+CHA film			-

DISCUSSION

Reduction on all three group at 21st and 28th on relative attachment loss and pocket depth and 7th, 21st, 28th of bleeding on probing were because all three group were treated with curretage treatment. This reduction related to inflammation reduction and new attachment formation on periodontal tissue after curretage treatment.

The highest pocket depth and relative attachment loss reduction on curretage + CHA film group at 21st (Table 1 I) in line with Komara dkk. (2) research with the result showed that gelatin carbonate apatite as an adjuvant can reduce pocket depth and relative attachment loss value significantly. Gelatin carbonate apatite film can increase fibroblast proliferation, kolagen, and amount of new vascular proliferation which lead to pocket depth and relative attachment loss reduction.

Highest pocket depth and relative attachment loss reduction on curretage + metronidazole group at 28th (Table I) because remodeling maturation phase on periodontal treatment after curretage take place after day 21st. At this phase peiodontal tissue is strenghten with colagen and elastin deposition. Collagens will form new cell bond and continuesly supporting tissue healing and reduce relative attachment loss and pocket depth (1).

Lower pocket depth and relative attachment loss reduction on curretage + CHA film-metronidazole group at 21st compared with curretage + CHA film group because metronidazole will become zwitterion on neutral pH. Metronidazole will have positif and negative ion (10). Hoare dan Kohane (11) also state that physical bond will formed on drugs combination. Positive ion N+ from metronidazole will interact with gelatin negative ion R-COO- so that metronidazole have stronger bond with carbonate apatite gelatin than carbonate apatite gelatin itself. This bond cause metronidazole release will be longer and increase its potential in periodontal tissue.

Highest bleeding on probing reduction on curretage

+ CHA film-metronidazole group at 7th (Table I) was because carbonate apatite which combined with gelatin in form of film was proved as temporary defect filler and wound dressing which can reduce bleeding on probing score (6, 7). Carbonate apatite gelatin film adjunction will also create new function as Drug Delivery System (DDS). This function will increase metronidazole therapeutic effect and make metronidazole concentration on tissue become more optimal and more stable from Gingival crevicular Fluid (GCF) and saliva flow in oral (4, 5, 9, 12).

CONCLUSION

The addition of carbonated apatite-gelatin film as a metronidazole delivery system provides a higher effect of reducing Relative Attachment Loss (RAL), Pocket Depth (PD), and Bleeding on Probing (BOP) compared to metronidazole in healing post-curettage chronic periodontitis. The highest reduction occurred in the application of apatite carbonate gelatin film alone.

ACKNOWLEDGEMENT

We would like to thank Universitas Gadjah Mada for funding this study through Program of Final Project Recognition (Rekognisi Tugas Akhir).

REFERENCES

1. Carranza FA, Newman MG, Takei HH, Klokkevold PR. Carranza's Clinical Periodontology. 12th ed. Louis Missouri: Saunders Elsevier. 2015: 9-35; 408-410; 493-495.
2. Komara I, Rusminah N, Hendiani I, Sopiatin S, Utami ND. The effect of Apatite Carbonate Membrane Application on Periodontal Tissue after Scaling and Root Planing Treatment. IJCRGG. 2018;11(09):162-9. DOI:10.20902/IJCTR.2018.110921.
3. Kuru L, Kuru B, Noyan Ь, Kьkrer A, Acar T, Yılmaz S. Effects of adjunctive local or systemic metronidazol with nonsurgical periodontal therapy on periodontal clinical parameters and gingival crevicular fluid biomarkers. Nobel Med. 2012; 8(1): 89-94.
4. Ardhani R, Setyaningsih S, Hafiyyah OA, Ana ID. Preparation of carbonated apatite membrane as metronidazole delivery system for periodontal application. Key Engineering Materials. 2016; 696: 250-8.
5. Hara K, Fujisawa K, Nagai H, Takamaru N, Ohe G, Tsuru K, Ishikawa K, Miyamoto Y. Fabrication and Physical Evaluation of Gelatin-Coated Carbonate Apatite Foam. Materials (Basel). 2016; 9(9):711: 1-9. doi: 10.3390/ma9090711.
6. Zhang J, Yao Y, Wei A, Shen L, Chen S. Synthesis, biodegradability, and drug delivery property of apatite/gelatin composite microspheres. In IOP Conference Series IOP Publishing: Materials Science and Engineering 2018; 439(4): 042009.
7. Foox M, Zilberman M. Drug delivery from gelatin-based systems. Expert Opin Drug Deliv. 2015;12(9):1547-63. doi: 10.1517/17425247.2015.1037272.
8. Won JE, El-Fiqi A, Jegal SH, Han CM, Lee EJ, Knowles JC, Kim HW. Gelatin-apatite bone mimetic co-precipitates incorporated within biopolymer matrix to improve mechanical and biological properties useful for hard tissue repair. J Biomater Appl. 2014; 28(8):1213-25. doi: 10.1177/0885328213502100.
9. Takahashi H, Misato K, Aoshi T, Yamamoto Y, Kubota Y, Wu X, Kuroda E, Ishii KJ, Yamamoto H, Yoshioka Y. Carbonate Apatite Nanoparticles Act as Potent Vaccine Adjuvant Delivery Vehicles by Enhancing Cytokine Production Induced by Encapsulated Cytosine-Phosphate-Guanine Oligodeoxynucleotides. Front Immunol. 2018; 9:783. doi: 10.3389/fimmu.2018.00783.
10. Ganjali MR, Norouzi P, Faridbod F, Pirelahi H. Novel Metronidazole Membrane Sensor Based on a 2, 6-(p-N, N-Dimethylaminophenyl)-4-Phenylthiopyrylium Perchlorate. J Chin Chem Soc. 2007; 54(1):55-61. https://doi.org/10.1002/jccs.200700010
11. Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. polymer. 2008; 49(8):1993-2007. https://doi.org/10.1016/j.polymer.2008.01.027.
12. Lцfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin Infect Dis. 2010; 50 Suppl 1:S16-23. doi: 10.1086/647939.