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Research Article

FORMULATION AND *IN VITRO* EVALUATION OF ALGINATE BASED METRONIDAZOLE PERIODONTAL GEL

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ABSTRACT

Objectives: The objectives of this study were to develop alginate based metronidazole periodontal gel for local microbes eradication in periodontal pocket, to study the effect of 0.5 and 1% Carbopol addition to alginate base on drug release and antibacterial activity of the gel, and to identify the effect of storage at room temperature for 3 months on chemical and physical stability of the gel.

Methods: The 25% metronidazole periodontal gel was prepared using alginate gel alone and in combination with 0.5 and 1% Carbopol. All of prepared formulations were evaluated for the pH, drug release, and antibacterial susceptibility. Stability of formulations were studied for 3 months including drug content, color and viscosity.

Results: The release of metronidazole from alginate gel showed sustained release properties. Within 300 minutes study, metronidazole released from formulations without Carbopol, with 0.5%, and 1% Carbopol were 40.17%, 47.67%, and 51.48%, respectively. Addition of 0.5 and 1% Carbopol did not significantly affect the drug release. All formulations showed excellent inhibition to *Staphylococcus aureus*. The largest zone of inhibition was found on formula without Carbopol, then followed by formula with 1% and 0.5% Carbopol. Storage at room temperature for 3 months caused slight degradation of metronidazole content and viscosity of all formulations. However, the color was not affected.

Conclusions: It is concluded that metronidazole periodontal gel can be formulated using alginate base. The alginate based metronidazole periodontal gel has sustained release properties. All formulations have excellent antibacterial activity, and stable on storage at room temperature for 3 months.

Keywords: periodontitis, periodontal gel, metronidazole, alginate, Carbopol

INTRODUCTION

Periodontitis is defined as an inflammation and progressive destruction of the tooth-supporting structures (periodontium) [1], which is caused by bacterial infection of a periodontal pocket accompanying with sub-gingival plaque [2]. The progression of periodontitis can be arrested by mechanical debri-dement consisting of scaling, root planing and proper oral hygiene control. However, pathogenic bacteria may not be eliminated in the deep periodontal pockets due to poor access for mechanical debridement, root anatomical complexity and the ability of the bacteria to invade and reside in the periodontal tissues or dentinal tubules [1].

The current microbiological treatment of periodontitis is through either the mechanical cleaning of the teeth by scaling and root planning along with systemic antibiotics or a localized delivery system incorpora-ting an antibiotic [3]. A prolonged administration of antibiotics raises a number of issue such as antibiotic resistance and adverse drug reactions like nausea, diarrhea and pseudomembranous colitis. Study showed that antibiotic concentration released in gingival crevicular fluid in periodontal pocket in sistemic use might be enough to inhibit bacteria in planktonik form, but not the ones within biofilms [4]. Large doses must be taken in order to achieve sufficient concentrations in the gingival crevicular fluid of the periodontal pockets; this brings with it the accociated side effects of antibiotics and problems regarding antibiotic resistance [3].

Because of these considerations, a variety of specialized local delivery systems are designed to maintain the antibiotic in the gingival crevicular fluid at a concentration higher than that achieved by systemic adminis-tration. Semi solid formulation (i.e., gel) has some advantages such as faster drug release, easier preparation, easier administration, higher biocom-patibility and mucoadhesivity, and rapid elimination through normal catabolic pathways [3].

Metronidazole is particularly suitable to be used as local antimicrobial treatment of periodontal diseases due to its restricted spectrum of activity against obligate anaerobes [4]. Marketed metronidazole gel available in Indonesia is mostly too bitter and too thin to be applied in periodontal pocket. Bitter taste of metronidazole causes numerous patient complaints. Thin formulation causes gel can easily spreads and runs out of periodontal pocket. Therefore, its effectivity in providing sufficient concentration of antimicrobial in periodontal pocket becomes not reliable.

Sodium alginate is a natural polymer, which is biocompatible and biodegradable. Bangun has reported that ointment with alginate base is highly hydrophil. It has pseudoplastic rheology and non-irritating properties [5]. Carbomers are synthetic high-molecular-weight polymers of acylic acid that are crosslinked with either allylsucrose or allyl ethers of pentaerythritol [6]. Carbopol (Carbomer) has been well-known as a bioadhesive agent for controlled release delivery system. Bansal, et. al., had used 1% Carbopol as mucoadhesive agent in local gel for periodontitis [3]. In this study, 0.5% Carbopol were used in formulation as comparation to 1% Carbopol to observe the effect of Carbopol addition to the drug release and antimicrobial activity of gel.

The main purpose of these study was to develop and evaluate metronidazole gel formulations using alginat base in the form of gel for local microbe eradication in periodontal pocket.

MATERIALS AND METHODS

Metronidazole obtained from Mutifa Ltd. Co., Medan, Indonesia, sodium alginate (Wako Pure Chemical Industries Ltd., Japan), Carbopol 974® P NF was a gift from Lautan Luas, Ltd. Co., Jakarta, Indonesia, potassium dihydrogen phosphate (Merck), sodium hydroxide (Merck), chloride acid (Merck), calcium chloride dihydrate (Merck), glycerol, nutrient agar (Oxoid), nutrient broth (Oxoid), sterile double distilled water (Ikapharmindo Putramas Pharmaceutical Laboratories, Ltd., Indonesia), stock culture of

Staphylococcus aureus obtained from Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara.

Preparation of Metronidazole Periodontal Gel

All of the formulations are shown on Table 1.

Table 1: Formulations of metronidazole periodontal gel

Materials	F1	F2	F3
Metronidazole (g)	25	25	25
Na alginate (g)	1	1	1
Ca chloride (g)	0.05	0.05	0.05
Glycerol (g)	50	50	50
Carbopol (g)	-	0.5	1
CO2-free distilled water (g)	ad 100	ad 100	ad 100

Every material was weighed accurately. Metronidazole was firstly finely grinded and the calcium chloride was dissolved in CO_2-free distilled water. Sodium alginate and Carbopol 974P were finely grinded and glycerol was slowly added into them until homogen mixture was obtained. Calcium chloride solution was slowly added to the mixture until viscous and stiff gel mass was formed. Fine powder of metronida-zole was added into the gel mass until homogen mixture obtained. The prepared gel was kept for 8 hours at room temperature before evaluations.

pH Determination

The pH of formulations was determined after diluting and dispersing them in distilled water (10% w/v). All measurements were made in triplicate and mean was calculated [7].

Drug Release Study

Metronidazole release from gel was studied using the method reported by Zuber [8] with some modifications. A dissolution apparatus consist of a 300 ml water jacket cylindrical glass as medium compartment, a glass pipe (length = 17 cm; diameter = 2,4 cm), magnetic stirrer, thermometer and thermostat was used.

Five hundred miligrams of gel was put on a 2.5 x 2.5 cm^2 cellulose acetate membrane. A piece of stainless steel wire netting was used as supporter to tie the membrane to one end of glass pipe. The water jacket cylindrical glass was filled with 250 ml phospate buffer pH 6.8. The pipe end tied with membrane was then immersed in cylindrical glass to a depth of ± 2.5 cm below the surface of phosphate buffer, agitated by a magnetic stirrer and temperature maintained at 37 ± 0.5°C throughout the study. Aliquots of 1 ml were withdrawn periodically at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min. The volume of the aliquots was made up to 25 ml with the fresh phosphate buffer. Each time of aliquots withdrawal, equal volume was replaced with fresh phosphate buffer previously heated to 37 ± 0.5°C. The amount of drug released was determined using UV-spectrophotometer at 320.0 nm.

Antibacterial Susceptibility Test

Aseptically prepared formula-tions of F1 to F3 were used in microbial assay. Inoculum was made by transfering *S. aureus* into 10 ml nutrient broth. The turbidity of the suspension was ajusted using visible spectrophotometer at 580 nm until 25% transmittance was obtained.

Sterile melted nutrient agar was cooled to 45°C. 0.1 ml *S. aureus* inoculum was put into a sterile petri disk and 20 ml agar was poured into it. The innoculated agar was well-mixed and left to solidify. Two wells (with ± 5 cm distance from each other) were bored using a sterile cork on the solid innoculated agar. The 0.1 ml blank and 0.1 ml gel solution that equals to 6.25 mg metronidazole were put into each well. The innoculated agar was incubated at 37°C for 24 hours, then zone of inhibition was measured. This was continued for 3 days and zone of inhibiton was measured on every 24 hours interval.

Stability Study

Stability of formulations was studied by keeping all the formulations at room temperature for 3 months. Formulations were analyzed for their drug content, color, and viscosity. Drug content analysis was conducted every 1 month interval for 3 months. Evaluation of the color and viscosity was conducted every 3 weeks interval for 12 weeks.

Drug Content Analysis

Fifty miligrams of gel was weighed accurately and dissolved in 25 ml 0.1 N HCl and filtered through No.1 Whatman paper. Filtrate was collected in 100 ml volumetric flask. The filter paper and residual was extracted with 0.1 N HCl and refiltered until the filtrate gave zero absorbance at 276.8 nm spectrophoto-metrically. The volume of all collected filtrate was made up to 100 ml with 0.1 N HCl (theoretical concentration: 125 ppm). 2 ml aliquots was withdrawn and put into 25 ml volumetric flask. The volume of aliquots was made up to 25 ml with 0.1 N HCl (theoretical concentration: 10 ppm). Its absorbance was spectrophotometrically measured at 276.8 nm. All measurements were made in triplicate and mean was calculated.

Color Evaluation

The change in color of formulations was visually observed.

Viscosity Study

The viscosity of formulations was studied using Brookfield viscometer with spindle number 64 at 0.3 revolutions per minute at room temperature. All measurements were made in triplicate and mean was calculated.

RESULTS AND DISCUSSION

Preparation of Metronidazole Periodontal Gel

All of the gel formulations prepared were highly viscous gel with yellowish white color, odorless, and syringable through 20 gauze needle. All formulations prepared were evaluated for the drug release, pH, antibacterial activity, and stability including drug content, color, and viscosity.

Glycerol is a simple poliol widely used in pharmaceutical formulations. Glycerol has sweet taste. Thus, in this conducted research, glycerol was used as the part of gel base in order to decrease the bitter taste of metronidazole. Sodium alginate and Carbopol were used to increase the viscosity of formulations to create thicker gel that can not easily run out of periodontal pocket.

Sodium alginate and Carbopol were used as high viscosity basis of gel. Thick formulation will enhance durability of gel to settle in periodontal pocket therefore it can increase its effectivity as local antimicrobial formulation.

pH Determination

Table 2: pH of formulations

Formulations	F1	F2	F3
	8	5.7	4.9
Determined	8	5.6	4.9
pH	7.9	5.6	4.9
Average	8	5.6	4.9

F1: without Carbopol; F2: added 0.5% Carbopol; F3: added 1% Carbopol

Table 2 shows that pH of alginate gel decreased by the addition of 0.5 and 1% Carbopol. Carbomer contain between 56-68% of carboxyclic acid (COOH) groups calculated on the dry basis [6], thus it is an acidic polymer. Increasing concentration of Carbopol can lower the pH of gel formulation.

Drug Release Study

In 300 minutes drug release study, it was shown that each F3, F2, and F1 was able to release 51.48%, 47.67%, and 40.17% metronidazole from gel formulation, respectively. Therefore, the drug release rate of F3>F2>F1. Statistical analysis based on the AUC (Area Under Curve) value of all formulations using ANOVA (*Analysis of variance*) in 95% confidence intervals showed that there was not any significant AUC value difference among three formulations. The addition of 0.5 and 1% Carbopol did not significantly affect the drug release of the gel.

Bangun has reported that there is a correlation between hydrophilic properties of the basis and drug release rate. When more hydrophilic basis is used in formulation, the water absorbtion rate become higher and so does the drug release rate [5]. Both alginate and Carbopol are highly hydrophilic polymers. Increasing concentration of hydrophilic polymer will increase the drug release rate. However, futher study about retention time of the gel in the periodontol pocket is needed.

Figure 1 shows the release of metronidazole from all formulations in phosphate buffer pH 6.8 at 37°C.

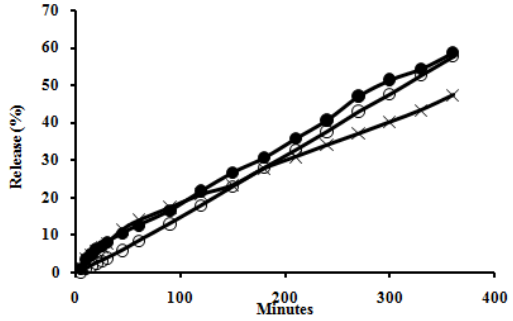

Figure 1. The effect of Carbopol addition on drug release from gel formulations in phosphate buffer pH 6.8 at 37°C (n=3).

Initial metronidazole amount in gel formulations was 125 mg

F1(✕): without Carbopol; F2(⊖): added 0.5% Carbopol;

F3(●): added 1% Carbopol

Antibacterial Susceptibility Test

S. aureus is a part of human normal flora. *S. aureus* is not a main periodontal pathogens that induce periodontitis. In this study *S.aureus* was only used as a model bacteria to prove the effectiveness of formulated gel in releasing the metronidazole content and killing bacteria.

The results of the antibacterial studies of all formulations against *S. aureus* using agar diffusion method are shown on Figure 2, and the diameter of zone of inhibition is shown on Table 3. All formulations showed excellent zone of inhibition after 1-3 days incubation at 37°C. However, there was no zone of inhibition on the blank. Statistical analysis on the zone of inhibiton of all formulations for 3 days observation period using ANOVA (*Analysis of variance*) in 95% confidence intervals showed that on the first, second, and third day of incubation, zone of inhibition among all formulations were significantly different. The zone of inhibition size were F1>F3>F2.

Table 3: The effect of Carbopol addition on metronidazole antibacterial activity against *S. aureus* incubated at 37°C for 3 days in nutrient agar media (n=3).

	Zone of inhibition (mm)		
Day	**F1**	**F2**	**F3**
1	35.70 ± 0.61	34.47 ± 0.12	35.20 ± 0.35
2	35.05 ± 0.18	33.93 ± 0.12	34.68 ± 0.26
3	34.97 ± 0.06	33.82 ± 0.10	34.30 ± 0.52

F1: without Carbopol; F2: added 0.5% Carbopol; F3: added 1% Carbopol

Davis and Stout has classified the strength of antimicrobial activity in agar diffusion method based on the zone of inhibition. If the zone of inhibition less than 5 mm, the inhibition activity is weak. If the zone of inhibition is 5-10 mm, the inhibition activity is moderate. If the zone of inhibition is 10-19 mm, the inhibition activity is strong. If the zone of inhibition is 20 mm or larger, the inhibition activity is excellent [9]. Antibacterial activity of all formulations against *S. aureus* in 3 days observation period was concluded as excellent. The strongest antibacterial activity was found on F1, then followed by F3, and F2, respectively.

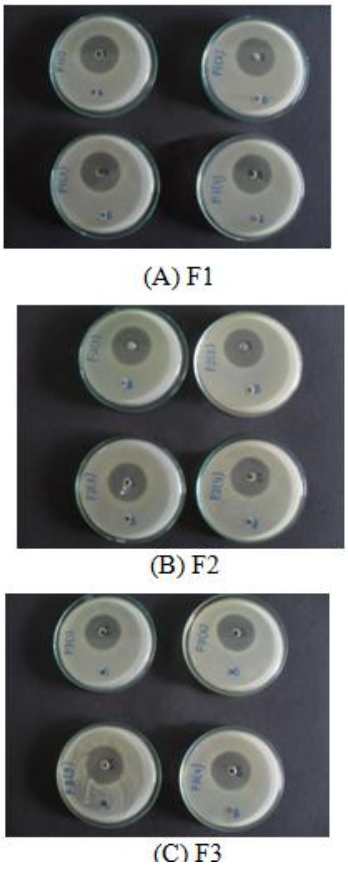

Figure 2: The zone of inhibition of metronidazole periodontal gel against S. aureus incubated at 37oC for the first 24 hours in nutrient agar.

F1: without Carbopol; F2: added 0.5% Carbopol; F3: added 1% Carbopol

Antibacterial activity of F3 was greater than F2, it might due to the pH difference, where pH of F3 was 4.90, and pH of F2 was 5.63. Lower pH of F3 supported antibacterial activity of metronidazole because optimum pH for the growth of S. aureus is around 6.0-7.0 [10]. However, F1 showed the best antibacterial activity by having the largest zone of inhibition. It might due to its viscosity which was the lowest among all formulations. Lowest viscosity of F1 might increase the diffusion rate of metronidazole through F1 basis.

However, further study is needed to understand the effect of formulated gel on main periodontal pathogens such as Aggregatibacterium Actinomycetem-comitans, Eikenella corrodens, Prevotella intermedia, and Bacteroides fragilis.

Stability Study

All gel formulations showed slight decrease of drug content, and viscosity by the time of storage for 3 months. However, there was not any color change observed.

Drug Content Analysis

Metronidazole gel monograph in USP 30-NF 25 contains not less than 90.0% and not more than 110.0% of the labelled amount of metronidazole [11]. Metronidazole content in all formulations, before and after 3 months storage at room temperature fulfilled the requirement of metronidazole gel monograph in USP 30-NF 25. The results of drug content analysis are shown in Table 4.

Table 4: The effect of storage at room temperature for 3 months on drug content of formulations

Month	Metronidazole content (%)		
	F1	F2	F3
0	109.76	97.88	96.95
1	103.77	95.69	95.05
2	100.81	93.26	94.99
3	99.04	91.06	94.77

Initial amount of metronidazole = 12,5 mg

F1: without Carbopol; F2: added 0.5% Carbopol; F3: added 1% Carbopol

Table 4 shows that the drug content of gel formulations decreased on storage, it might due to hydrolysis of metronidazol in formulations. It has been reported that metronidazole is stable in dry state when stored at room temperature [12]. However, metronidazole has also reported to undergo hydrolysis in aqueous media. Degradation process of metronidazole in aqueous solution follows pseudo first order kinetic [13].

Table 5 shows that the best stability was found on F3 followed by F2 and F1, respectively. This might due to pH difference among formulations. Average pH of F3 was 4.9. Then, average pH of F2 and F1 were 5.6 and 8.0, respectively. This result is in agreement with study conducted by Wu and Reza that overall, metronidazole was relatively stable under all the pH conditions [12]. Spesifically, pH 4.0 provided the most stable condition, while slight accelaration of the degradation was noticed in either pH 2.0 or 6.0 condition.

Table 5: Degradation rate constant (k) and T_{90} of metronidazole in gel formulations

Formula	k (month $^{-1}$)	T_{90} (month)
F1	0.03426	3.08
F2	0.02408	4.38
F3	0.00758	13.9

Calculated using first order kinetics equation

Color Evaluation

Based on conducted 12 weeks observation, there was not any color difference among formulations before and after storage.

Viscosity Study

The viscosity of gel was determined for formulations stored at room temperature for 12 weeks. The results are shown on Table 6. The viscosity of F3>F2>F1. Viscosity of F3 had not been measurable since pre-storage period until the twelveth week of storage, because its viscosity was higher than maximum viscosity measureable by Brookfield Viscometer, which is 20,000 poise. Viscosity of F2 was not measurable on the pre-storage period and third week of storage, but it was started to be measurable since the sixth week of storage. Viscosity of all formulations decreased by the time of storage. All of the gel formulations were syringable through 20 gauze needle.

Table 6: The effect of storage at room temperature for 12 weeks on viscosity of the formulations

Week	Viscosity (Poise)		
	F1	F2	F3
0	15,933	>20,000	>20,000
3	14,733	>20,000	>20,000
6	13,766	17,866	>20,000
9	12,600	17,100	>20,000
12	12,383	15,000	>20,000

F1: without Carbopol; F2: added 0.5% Carbopol; F3: added 1% Carbopol

Table 6 shows that viscosity of gel formulations decreased on storage. It might due to alginate degradation during storage period. Alginate, being a simple-stranded polymer, is susceptible to a variety of depolymerization processes. The glycosidic linkages are cleaved by both acid and alkaline degradation mechanisms and by oxidation with free radicals [14]. Free radicals degrade alginate mainly by oxidative-reductive depolymerization reactions caused by contamination of reducing agents like polyphenols from the brown algae [15,16,17]. Degradation can decrease molecular weight of alginate which will decrease its viscosity [18]. Alginate degradation has been evaluated by irradiation technique. It has been reported that degradation of alginate causes degradation of molecular weight and viscosity of alginate solution [19].

CONCLUSION

Metronidazole periodontal gel can be formulated using alginate alone and in combination with Carbopol. Addition of 0.5 and 1 % Carbopol into gel formulation did not significantly affect the drug release. All formulations showed excellent strength of inhibition against S. aureus with zone of inhibition > 20 mm. Storage at room temperature affected stability of all formulations which was shown by slight degradation of metronidazole and viscosity of all formulations.

Overall, the 25% metronida-zole periodontal gel formulations can slowly release their drug content and have excellent antibacterial activity.

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