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Research Report

Penetration effect of prostaglandin E_2 gel on oral mucosa of rats

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ABSTRACT

Background: *Several researches reported that Prostaglandin E_2 (PGE_2) injection on buccal mucosa combined with orthodontic pressure can faster tooth movement but has disadvantages such as high alveolar bone and root resorption furthermore pain from injection needle. PGE_2 gel was made to better replace the lacks of injectable PGE_2.* ***Purpose:*** *This research was aimed to prove that PGE_2 gel can penetrate rat's oral mucosa effecting the appearance of PMN cells.* ***Methods:*** *This research was an in vivo laboratory experiment using 36 Sprague Dawley rats which were divided into 3 groups: normal group, topical PGE_2 gel group after 1, 2, 4, 8 hours (4 subgroups), and topical gel without PGE_2 group after 1, 2, 4, 8 hours (4 subgroups). Each group consists of 4 rats, therefore the total sample for all research groups were 36 rats. Gel with 25 µg/mL of PGE_2 and gel without PGE_2 were applied on oral mucosa for 2 minutes. Then, the rats were sacrificed after 1 hour, 2 hours, 4 hours, and 8 hours application. After that, the samples were prepared for histological examination with Hematoxyllin and Eosin. The picture were taken with OptiLab View and PMN cells amount were counted with light microscope, set 400 times of magnification.* ***Results:*** *Penetration effect of PGE_2 gel on rat's oral mucosa result in PMN inflammation cells distribution. One-way ANOVA showed no significant difference on PMN cells count in rats' lower jaws between groups of normal and gel without PGE_2. There was significant difference between groups of PGE_2 gel and gel without PGE_2 ($p=0,001$). PGE_2 gel application showed PGE_2 as inflammatory media, even though administered topically.* ***Conclusion:*** *PGE_2 gel can penetrate rat's oral mucosa, effecting PMN cells 1, 2, 4 and 8 hours after application of PGE_2 gel.*

Key words: *PGE_2, PMN, tooth movement*

ABSTRAK

Latar belakang: *Beberapa penelitian melaporkan bahwa injeksi (Prostaglandin E_2) PGE_2 pada mukosa bukal yang dikombinasikan dengan tekanan ortodonti dapat mempercepat pergerakan gigi, tapi mempunyai kekurangan berupa resorpsi yang besar pada tulang alveolar dan akar gigi, serta adanya rasa sakit akibat penggunaan jarum suntik. Gel PGE_2 dibuat untuk mengatasi kekurangan pemberian PGE_2 secara injeksi.* ***Tujuan:*** *Untuk membuktikan bahwa gel PGE_2 dapat berpenetrasi pada mukosa mulut tikus dengan efek munculnya sel PMN.* ***Metode:*** *Jenis penelitian adalah eksperimental laboratorik in vivo, menggunakan 36 tikus Sprague Dawley yang dibagi menjadi 3 kelompok, yaitu kelompok normal; kelompok pengolesan gel PGE_2 setelah 1 jam, 2 jam, 4 jam, 8 jam (4 sub kelompok); kelompok pengolesan gel tanpa PGE_2 setelah 1 jam, 2 jam, 4 jam, 8 jam (4 sub kelompok). Masing-masing kelompok terdiri 4 sampel, sehingga total sampel seluruh kelompok penelitian 36 tikus. Gel PGE_2 dosis 25 µg/mL dan gel tanpa PGE_2 dioleskan pada mukosa mulut rahang bawah selama 2 menit. Tikus di sacrifice setelah 1 jam, 2 jam, 4 jam dan 8 jam pengolesan. Kemudian dibuat sediaan histologi dengan pewarnaan Hematoxylin dan Eosin. Foto preparat diambil menggunakan OptiLab View. Hitung jumlah sel-sel PMN menggunakan mikroskop cahaya dengan pembesaran 400x.* ***Hasil:*** *Efek penetrasi gel PGE_2 pada mukosa mulut terlihat distribusi sel-sel inflamasi PMN. Uji one-way ANOVA menunjukkan tidak ada perbedaan jumlah sel PMN yang bermakna pada mukosa rahang bawah tikus antara kelompok gel tanpa PGE_2 dan normal. Ada perbedaan bermakna antara jumlah sel PMN kelompok pengolesan gel PGE_2 dengan gel tanpa PGE_2. ($p = 0,001$). Hasil aplikasi gel PGE_2 menunjukkan gel PGE_2 sebagai media inflamasi, meskipun*

zat aktif diberikan secara topikal. ***Kesimpulan:*** *PGE_2 gel dapat berpenetrasi ke mukosa mulut tikus, dengan efek adanya sel-sel PMN pada 1 jam, 2 jam, 4 jam dan 8 jam setelah pengolesan gel PGE_2.*

Kata kunci: *PGE_2, PMN, pergerakan gigi*

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INTRODUCTION

The orthodontic treatment has the goal to achieve good occlusion. It needs relative longer treatment time than other kinds of dental treatment with mean 28.5–29 months.[1,2] The longer of orthodontic treatment, may increase the adverse effect, such as caries,[3] gingivitis, and root resorption.[4] There are several ways to shorten the treatment time, e.g. self ligating system of brackets,[5] electromagnetic usage,[6] surgical corticotomy,[7] and prostaglandin E2 (PGE_2) injection on buccal mucosa. The studies have shown that PGE_2 injection could accelerate the tooth movement 1.6–2 times faster than control.[8,9] That is why, PGE_2 injection becomes an alternative to enhance the tooth movement in order to shorten the orthodontic treatment time.

The study of PGE_2 was done on experimental animal with PGE_2 injection dose, in range of 0.1–10 µg/mL, in cycle of 2–3 weeks (21 days).[8] Although PGE_2 injection could enhance the tooth movement, there are adverse effects of over resorption on alveolar bone and tooth root, also pain during needle infiltration.[10] This pain may be caused by the needle usage, the infiltration depth, needle penetration,[11] and PGE_2 acted as inflammation trigger which could be painful.

To overcome those effects, it is needed to develop a new kind of PGE_2 in a form of gel. Gel has an advantage in simple usage. It could be applied on oral mucosa without pain,[12] and in sequence, so that the effect are expected to be better.[13] Carboxylmethylcellulose (CMC) are the chosen gel.[13–14] CMC is one of cellulose derivative, a natural structural polymer found in plants.[11] Physical properties of CMC are pH 2,5–3, white, fluffy, acidic,hygroscopic powder with a slight characteristic odour. Characteristics of CMC as a bioadhesive polimer are common component in bioadhesive dosage forms, unaffected by temperature variations, hydrolysis, oxidation and resistant to bacterial growth. CMC is known as one of mucoadhesive polymers which are capable of attaching to oral mucosa surfaces.[14] The dosage of PGE_2 gel is bigger than injection due to the thickness of mucosa, in order to have PGE_2 effect on the bones. In this study, the dosage of the PGE_2 is 25 µg/mL or 3 times dosage in sequence of 0 hour, 2nd hour, and 4th hour.

Orthodontic tooth movement means that a sustained force is directly delivered into tooth or teeth using orthodontic appliance. Orthodontic force along with increased vascular permeability and cellular infiltration, trigger inflammatory processes in the involved dental and paradental tissues. Neutrophil, lymphocyte and monocytes called as PMN cells were invade on the tissues, enhancing PGE_2 release which indirectly cause the elevation in PGE_2.[12]

PGE_2 is an inflammation stimulator to trigger the capillary vasodilatation that brings the acute inflammation where the amount of PMN cells increased.[15] PGE_2 in a form of topical application that could trigger tissue inflammation.[16] Microscopic observation shows that oral mucosa inflammation could be seen from capillary vasodilatation due to inter or extra cellular dilatation.[15,16] Higher amount of PMN cells could be found on acute inflammation tissue than mono nucleus cells, especially neutrophil cells that could be seen after 30-minute of application.[16] On chronic inflammation, mono nucleus cells, especially limphocyte, are higher than PMN leucocyte cells.[17] The purpose of this study was to prove that PGE_2 gel could penetrate into oral mucosa based on the observation of PMN cells –count in vivo in oral mucosa of rats.

MATERIALS AND METHODS

Thirty six rats of Sprague Dawley, under supervision of LITBANGKES RI vetenerinarian with criteria of male, 3 months old, 200–230 g, were in good condition to be studied. Thirty six rats were divided into 3 groups: 16 rats with PGE_2 gel application (experiment), 16 rats with CMC gel only (control), and 4 rats without any application (normal). Rats in normal group were used as a validity to rats in control group. This study had been approved by Ethical Commission of Faculty of Dentistry, University of Indonesia No. 117/Ethical Clearance/FKG UI/IV/2012.

PGE_2 gel was made recently before based on the preliminary study which consisted of 25 µg active PGE_2, 0.03 g CMC powder, and 0.97 ml aquabidest. CMC powder was crushed using mortar and pestle, mixed with aquabidest and PGE_2. CMC gel was made resenter paratus. Gel without PGE_2 was CMC gel without active PGE_2. PGE_2 gel and CMC gel, each consisted of 100 mg, were applied on mesial area of 46 buccal mucosa. Twenty five µg/mL CMC gel application was applied using cotton bud for 2 minutes with circular movement. Sixteen rats of experiment and 16 rats of control were applied in sequence of 0 hour, 2nd hour, and 4th hour. Rats were sacrificed after 1 hour, 2

hour, 4 hour, and 8 hour of gel application of each group consisted of 4 rats.

On the 1st day, PGE_2 gel was applied on 4 rats and CMC gel was applied on the other 4 rats. All of the rats were sacrificed after 1 hour of gel application. On the 2nd day, PGE_2 gel was applied on 4 rats and CMC gel was applied on the other 4 rats. All of the rats were sacrificed after 2 hour of gel application. On the 3rd day, PGE_2 gel was applied on 4 rats and CMC gel was applied on the other 4 rats at 0 hour and 2 hour. All of the rats were sacrificed after 4 hour of gel application. On 4th day, PGE_2 gel was applied on 4 rats and CMC gel was applied on the other 4 rats at 0 hour, 2 hour and 4 hour. The rats were sacrificed after 8 hour of gel application. Four rats in normal group were sacrificed on the 4th day.

Furthermore, the mucosa and the bone of oral tissue were cut transversal on the mesial area of mandible first right molar. Histological preparation was done on Histology Laboratory of Faculty of Medicine, University of Indonesia. The fixation used 4% paraformaldehid for 12 hours, demineralized using 10% EDTA in 7.5% polyvinylpyrrolidone solution on 4°C until soft. Samples were dehydrated using alcohol in sequence on 4°C, xylol alcohol, pure xylol, and paraffin xylol in room temperature, and then the tissue was cut with the thickness of ± 6 μm, and dyed with HE.[17]

The pictures were taken with OptiLab View on the areas with the most of inflammation cells. PMN cells were counted using light microscope with enlargement of 400. Callibration test was done on 10% of samples (4 samples between interobserver histological expert of Faculty of Medicine, University of Indonesia and researcher).

RESULTS

Interobserver reliability test was performed between histological expert of Faculty of Medicine, University of Indonesia and researcher on 10% of the total sample to count the amount of PMN cells. Unpaired t-test showed that $p = 0.423$, $p > 0.05$ and there was no significant difference which meant the reliability test was good.

The group of CMC gel application as a control compared to normal group was needed to confirm the validity. One-way ANOVA test was performed on normal to control group and the statistic result showed $p = 0.099$, which meant that there was no significant difference on PMN cells-count observed from the area of mandible mucosa of rats between control and normal group (Table 1).

One-way ANOVA test was performed in order to know the difference of the amount of PMN cells-count of control and experiment. The result showed that there was significant difference between experiment and control group with $p = 0.001$, $p < 0.05$ (Table 2). Histology examination result (HE) from each group after application of 1 hour, 2 hour, 4 hour, and 8 hour is presented on Figure 1.

All picture in Figure 1 showed Sprague Dawley's oral mucosa layers and the arrow focus on inflammation cells. The picture in experiment groups were A, C, E, G showed increase inflammation cells or PMN cells-count compare to their control in picture B, D, F and H. Picture I was normal Sprague Dawley's oral mucosa layer and also showed some inflammation cells.

Table 1. The different of amount PMN cells-count after 1 hour, 2 hours, 4 hours and 8 hours of topical application gel between control and normal group, using one-way ANOVA

Normal group	n	Control group	n	$\bar{X}$ ± SD	p
(6.25 ± 2.06)	4	> 1 hour application	4	5.25 ± 0.96	0.099
		> 2 hour application	4	7.75 ± 0.96	
		> 4 hour application	4	7.75 ± 0.96	
		> 8 hour application	4	6.25 ± 1.71	

Table 2. The different of amount PMN cells-count after 1 hour, 2 hours, 4 hours and 8 hours of topical application gel between experiment and control group, using one-way ANOVA

	n	Control	Experiment	p
		$\bar{X}$ ± SD	$\bar{X}$ ± SD	
> 1 hour experiment	4	5.25 ± 0.08	11.25 ± 1.500	p = 0.001*
> 2 hour experiment	4	7.75 ± 0.957	24.25 ± 2.875	
> 4 hour experiment	4	7.75 ± 0.957	27.00 ± 2.944	
> 8 hour experiment	4	6.52 ± 1.708	30.75 ± 3.948	

* $p < 0.05$ significant

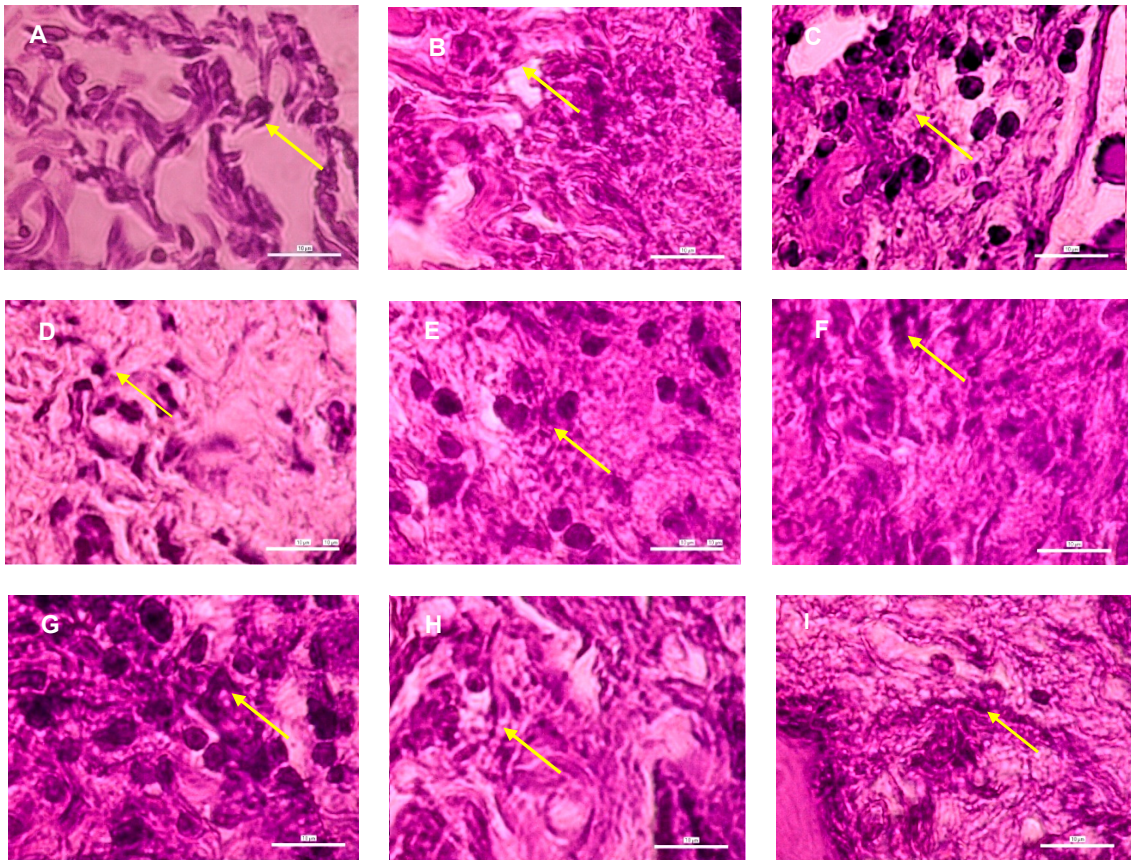

Figure 1. Oral mucosa on mesial area of 46 buccal's Sprague Dawley using light microscop with Opti Lab View, 400× magnification.
Arrow showed inflammation cells. (A) 1 hour after topical application PGE_2 gel; (B) 1 hour after topical application CMC gel; (C) 2 hour after topical application PGE_2 gel; (D) 2 hour after topical application CMC gel; (E) 4 hour after topical application PGE_2 gel; (F) 4 hour after topical application CMC gel; (G) 8 hour after topical application PGE_2 gel; (H)4 hour after topical application CMC gel, (I)Normal oral mucosa layer.

DISCUSSION

Several studies showed that PGE_2 injection on buccal mucosa combined with orthodontic force could enhance tooth movement, although it has a disadvantage of over root resorption, over resorption of alveolar bone, also a pain due to needle infiltration.[8] To overcome those effect, it is needed to develop a new kind of PGE_2 in form of gel. Gel has an advantage in simple usage in oral mucosa without pain.[13] CMC gel are the chosen gel, because of it's stability on storage, good tolerance of water miscible solvents and good adhesive strength.[14] In dentistry, until recently there is no PGE_2 gel.

CMC is known as one of mucoadhesive polymers which are capable of attaching to oral mucosa surfaces. Nowadays it has been accepted as a strategy of specific localization of drug delivery system on mucosa buccal area. Advantages associated with buccal drug delivery have rendered this route of administration useful for a variety of drugs.[14] PGE_2 gel was made by mixing CMC gel with PGE_2 as the active agent. CMC gel is a media for PGE_2 to penetrate into rats oral mucosa layer.

The purpose of this study was to examine the penetration effect of PGE_2 gel on experimental rats mucosa, as an inflammation mediator. Active agent PGE_2 is an inflammatory stimulator to trigger the capillary vasodilation that brings the acute inflammation where PMN cells increase.[16] PMN number are an indicator of the degree of acute inflammation. To assested the quantifying their number in tissue section usually used standardized system, called PMN cells-count.[17] If PGE_2 is given topically on human body, non-specific immunity response will appear, such as neutrophil, basophil, and macrofag as PMN and MN cells.[15] The application of active PGE_2 gel showed that PMN cells were increased to submucosa layer (Figure 1).

Rats oral mucosa structure is not different from epithel layer of human oral mucosa, but the thickness of rat's oral epithel is less than human, about 40–140 μm.[18] To be able to make the small dosage of PGE_2, which is 25 μg and could penetrate into mucosa layer, the application of PGE_2 gel could be done in sequence, 3 times of 0, 2, and 4 hours. The active accumulation could continue to penetrate into deeper mucosa tissue until the alveolar bones.

In this research, histological preparation was using Hematoxyllin and Eosin (HE) because it could show the inflammation tissue and the morphology of PMN cells clearly.[19,20] Futhermore, PMN cells-count could be done through light microscope pictures and this slide were photographed by using Opti Lab View with 400 magnification.

There are two main tissues component of the oral mucosa that consist of a stratified squamous epithelium, called the oral epithelium, and an underlying connective tissue layer, called lamina propice. Lamina propria is composed a connective tissue with several different cells: fibroblasts, macrophages, and inflammatory cells. Between lamina propria and alveolar bone there are submucosa layer.[15] HE staining showed that distribution of PMN cells were in submucosa layer, and the nucleus of the PMN cells appeared more red with violet colour. In Figure 1, especially on experiment group, shown the it amount of PMN cells higher than control group. It means that PGE_2 gel as stimulatory mediator could penetrate into deeper oral mucosa layer.

The control group was analyzed with one-way ANOVA test compared to the normal group, and showed that there was no significant difference between them (Table 1). It showed that the pressure during application could increase the PMN cells on control group; but it did not affect on endogenous formation of PMN cells on rats mucosa. So control group had a good validity as compared to the experiment group.

PGE_2 is derived from 20-carbon essential fatty acids that contain three, four or five double bounds.[20] PGE_2 is an inflammation stimulator that derived from arachidonat acid. PGE_2 is not stored on tissue but will be synthesized after the stimulation.[20,21] Topical application of PGE_2 could cause inflammation. Inflammation is controlled by the presence of a group of substances called chemical mediator such as vasoactive amine histamine, serotonin, kinin, fibrinolytic system, complement system and arachidonic acid (prostaglandin and leukotrienes). Vasoactive amine histamine is important in the initiation of early phase of acute inflammation as it mediates to increased vascular permeability. Some chemical mediator are interrelated inducing arteriole dilatation, fibrinolytic system produce plasmin. Plasmin does important things in inflammation. It can produced vasodilatation by generating fibrinopeptides.[16] This condition will trigger on acute inflammation cells.[15] That's why on group with active PGE_2 the amount of PMN cells were increase, compared to the control (Table 2 and Figure 1). This result showed that after 1 hour of PGE_2 gel application the mediator of inflammation increased even though the active agent was given topically. Based on the inflammation theory, inflammation process on the tissue had started on 30 minutes after stimulation.[16]

In acute inflammation there is a reactionary response by immune system. The important factors in acute inflammation acted by granulocyte cells included netrofil, eosinofil, basofil which called as PMN cells, some antibody and others complement. Histological examination result in this research showed that PMN cells were increased. Increased PMN cells-count was positive, because it proved the effect of PGE_2 as an inflammatory agent could penetrated into rats oral mucosa using gel as a media.

This study reports that PGE_2 gel could penetrate into rats oral mucosa based on PMN cells-count through of infammation process. For the next study we suggest to examine penetration effect of PGE_2 gel on rats oral alveolar bone.

It is concluded that PGE_2 gel could penetrate into rats oral mucosa based on the observation of PMN cell-count. After 1 hour, 2 hours, 4 hours, and 8 hours of PGE_2 gel application, there was a significant difference increasing of the PMN cells-count compared to control.

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