

Research Report

Topical applications effect of casein phospho peptide-amorphous calcium phosphate and sodium fluoride on salivary *Mutans Streptococci* in children

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ABSTRACT

Background: Dental caries is one of the major human diseases caused by *Mutans Streptococci* (MS). Topical application casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) dan Sodium fluoride are often use in children and play a role in the caries prevention. **Purpose:** The aim of study was to determine the effect of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) and Sodium fluoride topical applications to the number of salivary MS colonies in children. **Methods:** This study using cross-over design with quase experiment time-series. The subjects were 30 children in range of age 6-12 years old that obtained with simple random sampling. The saliva samples of subjects were collected 3 times. First, saliva samples were taken before the treatment; second, after CPP-ACP topical application; third, after sodium fluoride topical application. Between the CPP-ACP and sodium fluoride treatments there was a one week wash-out period. After each treatment, saliva samples were taken twice, 15 and 30 minutes after topical applications respectively. After cultivation on specific agar, the colony number of salivary MS was determined by colony counting (Colony Forming Units-CFU). **Results:** There was no significant difference between topical application casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) and Sodium fluoride in reducing the number of *Streptococcus mutans*. But topical application of Sodium fluoride tended to show more reduction than CPP-ACP. **Conclusion:** The topical application of CPP-ACP and Sodium fluoride could reduce the number of salivary MS in children. The effect of Sodium Fluoride was somewhat greater than CPP-ACP.

Key words: Casein phosphopeptide–amorphous calcium phosphate, sodium fluoride, *Streptococcus mutans*, saliva, children

ABSTRAK

Latar belakang: Karies gigi merupakan salah satu penyakit manusia utama yang disebabkan oleh *Streptococcus Mutans* (MS). Topikal aplikasi kasein phosphopeptide - amorf kalsium fosfat (CPP-ACP) dan sodium fluoride sering digunakan pada anak-anak dan berperan dalam pencegahan karies. **Tujuan:** Penelitian ini bertujuan untuk mengetahui pengaruh aplikasi kasein phosphopeptide - amorf kalsium fosfat (CPP-ACP) dan Sodium fluoride secara topikal terhadap jumlah koloni MS pada saliva anak. **Metode:** Penelitian ini menggunakan desain cross-over dengan percobaan Quase time-series. Subjek penelitian ini adalah 30 anak dalam rentang usia 6-12 tahun yang diperoleh dengan simple random sampling. Sampel saliva subjek dikumpulkan 3 kali. Pertama, sampel saliva diambil sebelum perlakuan; kedua, setelah aplikasi topikal CPP-ACP; ketiga, setelah aplikasi topikal sodium fluoride. Antara aplikasi CPP-ACP dan sodium fluoride ada periode wash-out satu minggu. Setelah perlakuan, sampel saliva yang diambil dua kali, 15 dan 30 menit setelah masing-masing aplikasi topikal. Setelah ditumbuhkan pada media agar yang spesifik, jumlah koloni MS ditentukan dengan cara hitung koloni (Colony Forming Unit-CFU). **Hasil:** Tidak ada perbedaan yang signifikan antara topikal aplikasi kasein phosphopeptide-amorf kalsium fosfat (CPP-ACP) dan sodium fluoride dalam mengurangi jumlah *Streptococcus mutans*. Tetapi aplikasi topikal Sodium

fluoride cenderung menunjukkan penurunan lebih banyak dari CPP-ACP. **Simpulan:** Aplikasi topikal dari CPP-ACP dan Sodium fluoride dapat mengurangi jumlah MS pada saliva anak. Pengaruh Sodium Fluoride sedikit lebih besar dari CPP-ACP.

Kata kunci: Casein phosphopeptide -amorf kalsium fosfat, sodium fluoride, *Streptococcus mutans*, saliva, anak

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INTRODUCTION

The prevalence of dental caries in children remains a significant clinical problem. In 2004 the caries rate in Indonesia is 90.05% of population, and in 2011 the most significant incidence occurs in children aged 3-5 years (81.2%) which mean most of the case are left untreated.^{1,2} So in Indonesia with population more than 225 million people the prevention is at top priority. Therefore, prevention efforts should be made as early as possible using simple and effective dental caries prevention method which affordable by all levels of society.

Topical application of sodium fluoride is widely used as dental caries prevention. Fluoride compound has been applied extensively, and its efficacy has been recognized by researchers and dentists. Besides of sodium fluoride recently there are materials that have role in preventing dental caries. One of them is the agent that has the *casein phosphopeptide- amorphous calcium phosphate* (CPP-ACP). Several studies on CPP-ACP showed not only the increase of enamel remineralization, but also prevent bacterial adhesion of *Streptococcus mutans* on tooth surfaces.³ The aim of study was to determine the effect of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) and Sodium fluoride topical applications to the number of salivary *Streptococcus mutans* colonies in children.

MATERIALS AND METHODS

This study using cross-over design with quase experiment time-series. The subjects were 30 children in range of age 6-12 years old that obtained with simple random sampling. Oral hygiene examination in children aged 6-12 years were done by using the OHI-S index with the criteria sample of very bad OHI-S; did not have allergies or certain systemic diseases; and were not taking antibiotics. Sampling was conducted in SDI Tamalanrea of Universitas Hasanuddin, while laboratory procedure was conducted at the Microbiology laboratory Faculty of Medicine Universitas Hasanuddin. The saliva samples of subjects were collected 3 times. First, saliva samples were taken before the treatment; second, after CPP-ACP topical application; third, after sodium fluoride topical application. Between the CPP-ACP and sodium fluoride treatments there was a one week wash-out period. After each treatment, saliva samples were taken twice, 15 and 30 minutes after

topical applications respectively. After 10^{-3} dilution, the saliva samples were cultured on Glucose Nutrient Agar (GNA). After the anaerob incubation 37°C for 24 hours, the colony number of salivary MS was determined by colony counting (Colony Forming Units-CFU).

Topical application of CPP-ACP (first treatment) and topical application of sodium fluoride (second treatment) were given to subjects with following manner: (a) dry the entire surface of the tooth samples by using cotton or cotton pellet; (b) topical application of CPP-ACP (GC Tooth Mousse®-Recaldent) approximately 1 mg and 0.5 ml Sodium fluoride 5% (Floucal solute®- Septodont) were applied to the entire surface of the teeth; (c) after 15-minute the application of topical material, the second saliva collection was carried out; (d) 30 minutes later, the third saliva sample was taken; (e) all saliva samples were brought to the laboratory for evaluation of the number of MS colonies. Data obtained by the calculation of the bacteria and then noted in table form and subsequently statistically tested by using ANOVA and t-test pairs.

RESULTS

The subject distribution was 18 males (60%) and 12 females (40%) with mean age was 8.56 ± 2.02 years. The value of oral hygiene index was 3.53 ± 0.38 (Table 1). Topical applications of CPP-ACP and sodium fluoride showed the significant reduction of salivary Mutans Streptococci colonies in 15 and 30 minutes after treatment respectively (Table 2), based on ANOVA test results, the value of $p = 0.000$ ($p < 0.05$). The further test results for the number of salivary *Mutans Streptococci* colonies by interval of time after the intervention of topical application of CPP-ACP and sodium fluoride materials described the differences of each bacterial colony count (Table 3). The colonies number of MS from the pretest to 30 minutes after of application topical of CPP-ACP and sodium fluoride decreased up to approximately 45 colonies. In addition, the decrease of colonies number based on each intervals showed significant results.

In the CPP-ACP group of materials, the number of colonies decreased to 40.00 CFU/ ml, whereas the number of colonies sodium fluoride group decreased to 35.73 CFU/ ml. The result of statistical tests showed $p = 0.214$ ($p > 0.05$), it means that the difference was not significant (Table 4). Meanwhile, after 30 minutes Sodium fluoride

Table 1. Characteristics distribution of the research sample

Characteristics of research sample	Frequency (n)	Percentage (%)	Mean \pm SD
Sex			
Male	18	60	
Female	12	40	
Age			8.56 \pm 2.02
Value of oral hygiene (OHI-S)			3.53 \pm 0.38
Number of colonies of <i>S. mutans</i> before (Pre-test)			83.40 \pm 22.63
CPP-ACP group	30	100	
Number of colonies in 15 minutes (1 st Post-test)			40.00 \pm 9.57
Number of colonies in 30 minutes (2 nd Post-test)			18.73 \pm 9.87
Sodium fluoride group	30	100	
Number of colonies in 15 minutes (1 st Post-test)			35.73 \pm 9.83
Number of colonies in 30 minutes (2 nd Post-test)			12.93 \pm 7.86

Table 2. The differences effect between CPP-ACP and sodium fluoride based on times interval

Topical application material group	CFU Pre-test	CFU 15 minutes	CFU 30 minutes	p-value
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
<i>CPP-ACP</i>	83.40 \pm 22.63	40.00 \pm 9.57	18.73 \pm 9.87	0.000*
<i>Sodium fluoride</i>	83.40 \pm 22.63	35.73 \pm 9.83	12.93 \pm 7.86	0.000*

*Repeated analysis of variance (ANOVA) test: $p < 0.05$; significant

Table 3. Further test results based on the number of colonies of *S. mutans* time intervals after giving topical application materials CPP-ACP and sodium fluoride

Topical application material type	CFU <i>S. mutans</i>	Comparator	Mean difference	p-value
CPP-ACP	CFU pre-test	CFU 15 minutes	43.400	0.000*
		CFU 30 minutes	64.666	0.000*
Sodium fluoride	CFU 15 minutes	CFU 30 minutes	21.266	0.000*
		CFU pretest	47.666	0.000*
	CFU 15 menit	CFU 15 menit	70.466	0.000*
		CFU 30 menit	22.800	0.000*

*Pos Hoc Test: Least Significant Difference (LSD) test: $p < 0.05$: significant

Table 4. The differences effect of topical application of CPP-ACP and Sodium fluoride materials in time intervals 15 minutes and 30 minutes after treatment

Topical Application Material group	CFU Pre-test	CFU 15 minutes	p-value	CFU 30 minutes	p-value
	Mean \pm SD	Mean \pm SD		Mean \pm SD	
CPP-ACP	83.40 \pm 22.63	40.00 \pm 9.57	0.214**	18.73 \pm 9.87	0.611*
Sodium fluoride	83.40 \pm 22.63	35.73 \pm 9.83		12.93 \pm 7.86	

*Paired sample t-test: $p > 0.05$; not significant

materials reduced the colony number of MS to 12.93, while the material of CPP-ACP reduced to 18.73. Based on the result of statistical tests, the value of $p = 0.611$ ($p > 0.05$), which means that there was no significant difference.

DISCUSSION

The study used topical application of GC Tooth Mousse product of Recaldent® which contain derivate calcium phosphate compound or CPP-ACP that have been applied for approximately 1 mg. The study used container of topical application because it has already tested and reported by several literatures. Dr. Santosh⁴ from India stated that consuming products that had the same anti-caries CPP-ACP can give good effect on controlling caries at the age of children and adults. As for the comparison the study used 0.5 ml Floucal solute -Septodont® that contain sodium fluoride compounds. It is based on the results of research on the role of fluorine reduces the ability of bacteria to form acid. In addition it also functions in the formation of fluorine mineral that will stop the caries process.^{4,5}

The result showed the difference in the colony number of MS after topical treatments which were given to subjects after 15 and 30 minutes. The results between each treatment compared with each other to analyze which one was more effective in reducing the number of colonies of *S. mutans*. The data revealed that both topical application materials were indeed effective in reducing the number of *S. mutans*.^{4,5,6}

Research by Reynolds in 2006 attracted public attention by revealing the fact that phosphopeptida casein amorphous calcium phosphate, which was one of the derivatives casein was able to get into the enamel surface and affect the caries process. CPP-ACP prevents the vicinity of MS to the tooth surface. Phosphopeptida bond casein (CPP) containing sequence group nano-complex chain was Ser (P)-Ser (P)-Ser (P)-Glu-Glu had an ability to prevent bacteria. Phosphopeptida casein chain arrangement (CPP) binds to amorphous calcium phosphate (ACP) which can prevent the development of bacteria.^{4,6,7}

In addition to preventing the vicinity of MS, CPP-ACP also assist in the remineralization of tooth enamel. The effectiveness of a paste containing 10% CPP-ACP on the enamel surface in vitro, revealed that 10% CPP-ACP has a positive effect on enamel remineralization. Other research suggests the use of CPP-ACP with 0.1% mg/ ml significantly reduced caries activity by 14%, whereas the levels of CPP-ACP with 1% mg/ ml could reduce 55% of caries activity.^{8,9}

Effectiveness of topical application of fluoride in reducing the number of colonies of MS has also been proved in the literatures and research. Fluoride works by inhibiting the metabolism of plaque bacteria that can ferment carbohydrates through changes hydroxyl apatite in enamel to fluoride apatite. Fluorine chemical reactions: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + \text{F} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OHF})$ (OHF)

produces the enamel more resistant to acid that can inhibit demineralization and enhance remineralization processes that trigger repair and termination of carious lesions.^{1,10}

This study also showed a difference in the effectiveness of topical application of CPP-ACP materials and Sodium fluoride at time intervals of 15 minutes and 30 minutes after give the materials. At 15 minutes after the intervention materials, group topical application of CPP-ACP materials, number of colony decreased to 40.00 CFU/ ml, while in the topical application of sodium fluoride materials decreased to 35.73 CFU/ ml. After 30 minutes, the topical application of CPP-ACP materials capable of reducing the number of colonies to 18.73 CFU/ ml, whereas topical application of sodium fluoride materials have reduced to 12.93 CFU/ ml. This shows that there is no significant difference between the effects of topical application of CPP-ACP materials and Sodium fluoride to decrease the number of colonies of *S. mutans* in the 15th minutes and 30 after give topical materials.

The study suggested that the two materials are effective in lowering the number of colonies of *S. mutans*, but the topical application of sodium fluoride materials showed a greater decrease compared with CPP-ACP materials at intervals of 15 and 30 minutes after give topical material in saliva children aged 6-12 years. However, there was no significant difference between topical application of CPP-ACP and Sodium fluoride at 15 and 30 minutes after providing topical material. Fluoride when consumed in large amounts can give side effects to the body, i.e. acute toxicity, as well as the occurrence of fluorosis (mottled enamel). It is therefore not recommended for use at home, and would be better if applied by professionals in order to prevent that side effect.^{10,11} While topical application of CPP-ACP material is derived from casein (milk) safe for children.

The study suggested that topical application of CPP-ACP and Sodium fluoride could reduce the number of salivary MS in children. The effect of Sodium fluoride was somewhat greater than CPP-ACP.

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