ADDITIONAL OF CHEMICAL ENHANCER FOR INCREASING THE PENETRATIONAL FLUORIDE MOUSE SKIN

(PENAMBAHAN BAHAN KIMIA UNTUK PENINGKATAN DAYA TEMBUS ION FLUORIDA PADA KULIT TIKUS)

Diyah Fatmasari*, Iwan Dwi Prahasto, Akhmad Kharis Nugroho***, Widjijono******

* Department of Dental Health
Health Polytechnic of Semarang, Faculty of Dentistry Unissula Semarang
** Department of Pharmacology
Faculty of Medicine Gadjah Mada University
*** Department of Biopharmaceutical
Faculty of Pharmacy Gadjah Mada University
**** Department of Biomaterial
Faculty of Dentistry Gadjah Mada University
Jl. Taman Sekar Jagad 24 Tlogosari Semarang 50196

Abstract

Fluoride plays an important roles in reducing dental caries by improving remineralization process and strengthening email through forming fluoroapatite which is more resistant to acid. Fluoride systemic mode without passing metabolism with small and controlled dose need to be developed such as Transdermal route. The aims of this research was to find if fluoride solution with and without enhancer solution is able to penetrate to skin. Quasy experimental design with post test only control group design as research approach was used. Transport test with Franz Like Diffusion cell used as the instrument in vitro skin permeation test with hairless and full thickness mouse skin as membrane between donor and recipient cell. Two groups of donor cell was fluoride solution and fluoride added with chemical enhancer: oleic acid and iso propyl alcohol (IPA) solution and recipient solution was CMF PBS 0,1 M pH 7,4. Control group was oleic acid solution. Sample was taken for time interval of 4, 20 and 24 hours and Fluoride containt was measured by Potensiometer Spesific Ion Fluoride. The results showed that there was an influence of transport test both on NaF solution and NaF+oleat acid and IPA solution (p= 0.00) and (0.00) on fluoride permeation, however there was no significant difference on control group (p= 0.07). NaF added with chemical enhancer solution and it had higher penetrating power than other solution. It can be concluded that added chemical enhancer can increase the penetration of fluoride.

Key words: fluoride permeation, NaF, chemical enhancer

Abstrak

Fluorida memegang peran penting dalam mengurangi kejadian karies gigi dengan meningkatkan proses remineralisasi dan memperkuat email melalui pembentukan fluoroapatite yang lebih tahan terhadap asam. Fluorida tanpa melewati jalur sistemik dengan dosis kecil dan terkontrol perlu dikembangkan misalnya rute transdermal (melewati kulit). Tujuan penelitian ini adalah untuk mengetahui apakah fluorida dalam bentuk larutan mampu menembus kulit. Desain penelitian adalah kuasi eksperimental dengan *post test only control group design* sebagai rancangan penelitian yang digunakan. Uji transpor dengan Franz *like Diffusion Cell* digunakan sebagai alat dalam uji permeasi kulit secara *in vitro* dengan kulit tikus sebagai membran pemisah antara sel donor dan sel penerima. Dua kelompok sel donor adalah larutan NaF dan larutan NaF ditambah dengan bahan kimia peningkat daya tembus yaitu asam oleat dan iso propil alkohol (IPA). Sebagai larutan penerima adalah PHM PBS 0,1 M pH 7,4. Kelompok kontrol adalah larutan asam oleat. Sampel diambil dalam jangka waktu 4, 20 dan 24 jam. Berat fluorida diukur dengan potensiometer Ion Fluorida Spesifik. Hasil penelitian menunjukkan ada pengaruh lama uji transpor pada larutan NaF dan NaF + asam oleat dan IPA solusi (p = 0,00 dan 0,00) terhadap daya tembus ion F, namun tidak ada perbedaan signifikan pada kelompok kontrol (p = 0,07). NaF ditambahkan dengan larutan kimia peningkat daya tembus (*chemical enhancer*) dan memiliki daya tembus yang lebih tinggi dibanding larutan tanpa *enhancer*. Sebagai kesimpulan, penambahan bahan kimia peningkat daya tembus dapat meningkatkan daya tembus ion fluorida pada kulit tikus.

Kata kunci: daya tembus fluorida, NaF, bahan kimia peningkat daya tembus

Edisi Cetak Dentika Dental Journal, Juli 2012 (ISSN: 1693-671X)

INTRODUCTION

Dental caries is a disease involves in multi combination factorial aspects of dietary carbohydrates and bacteria in the oral cavity, such as Streptococcus mutans and Lactobacillus.[1] The new theory of caries states the balance of the minerals structure in email is determined by the balance of the process of demineralization and remineralization that occurs in the oral environment. Each time sucrose attached biofilm, the bacteria will convert the sugar into acid, increase solubility of enamel to acid. [2]

Among several caries preventive strategies, the use of fluoride has been proven to be effectively reducing the incidence of caries based on clinical trials, literature reviews and meta-analysis involving in various fluoride preparations.[3,4] Current research recommends the use of fluoride in low dose, therefore, fluoride will release slowly is more effective than the use of topical application.[1]

Due to some shortcomings of the available various preparations of fluoride, it should be considered that an alternative route that is more convenient for patient in terms of application and can achieve the desired therapeutic dose.[7,8] Transdermal drug delivery system (TDD) is the route which drug released from the dosage form has to pass through the skin layers by a multistep sequential process before it reaches systemic circulation. The step includes diffusion through the lipophilic stratum corneum (SC), this is the most barrierest way for transdermal transport of drug molecules.

Not all drugs can be used in this way because there are several requirements that must be satisfied to pass SC. The molecular weights of the drug should be less than 500 daltons, hidrophilic and lipophilic characteristic (log P: 1-3), melting point below 200^0 C and dose less than 10 mg/day.[9]

Fluoride compounds are commonly used in the caries prevention is sodium fluoride (NaF) which has molecular weight of 41.988, solubility in water 4.13 g/100 g and usually dose in mg/day. Although there are some requirements that are not encountered the high melting point (993^0 C) and insoluble in lipid, but current technology can overcome these obstacles with the help of several ways through the media to penetrate to skin barrier,[10] for example by the addition of chemical enhancer.

The mechanism of enhancers is by enhancing drug percutaneous penetration may either disrupt lipid organization or interact with keratin in corneocytes.[10] Several transport-enhancing ingredients such as Propylene Glycol (PG), Iso Propyl Alcohol (IPA) which are used by [11] or d-limonene [12]. Permeability-enhancing ingredient that is often used because its low skin sensitivity and biocompatibility to hydrophilic drug is oleic acid.[13] A researcher used the combination of oleic acid and IPA as enhancer and is proven effectively increasing of Papaverine hydrochloride.[14]

This study aimed to know the possibility of fluoride to penetrate to mouse skin and can the chemical enhancer increase the penetration rate.

MATERIALS AND METHODS

Quasy-Experimental design with Post Test Only Design approach was used. The material was 500 ml solution of phosphate buffer (calciummagnesium free phosphate buffer solution, CMF-PBS) 0.1 M pH 7.4 used as medium transport solution and the sample was 5 ml of phosphate buffer solution pH 7.4 (CMF-PBS) after a per-meation test of time interval 4, 20, 24 hours. De-pendent variable of this study was contact time of NaF, NaF+ oleic acid and IPA and control solution into mouse skin on transport test while independent variable was the amount of fluoride permeation.

Data collection started with donor solution preparation. Three donor solutions were prepared in this research: NaF solution, NaF+oleic acid and IPA solution and oleic acid solution as a control. Method of making NaF solution: NaF powder 30 mg was weighed using a digital balance, then added 3 ml aquabidest in a plastic tube, and the solution was stirred until homogeneous and the concentration was 1000 ppm. Preparation NaF+enhancer was 300 mg NaF powder added 0,3 ml oleic acid, 0,3 ml Iso Propyl Alcohol (IPA) and 2,4 ml aquabidest. The third solution contained 3 ml oleic acid.

Next step was mouse skin preparation. The mouse was sacrificed and dissected its skin with electric shaver; lipid tissue under the skin was cleansed, the skin tissue was cut using a mal (mold) that has been adjusted by means of cell transport. Skin tissue was stored in sealed plasticcontainers that was given a solution of PBS, stored in a refrigerator until used.

In vitro permeation study was then implemented after that. Modified Franz diffusion like cell was used for the release and permeation study of NaF solution. Donor compartment contained of a concentration of 1000 ppm NaF solution and sealed. Membrane separation between donor and recipient compartments was mouse skin with a diameter of 1.25 cm, skin thickness of 0.1 mm. Membrane was placed between donor and recipient compartment with the dermis side facing the recipient compartment. Recipient compartment contained of 20 ml PBS pH 7.4 and continuously stirred using a magnetic bead with a speed of 50 rpm at room tem-

perature. At each predetermined time interval of 4, 20 and 24 hours, 5 ml of sample solution was taken using volume pipe from the right neck of the cell. After sampling, the opposite side of the neck cell was refilled with a solution of PBS pH 7.4 the same volume of sample taken. The samples then brought to BATAN for fluoride content analysis by Potensiometer specific ion fluoride. Factorial ANOVA test was used with time test transport factors to determine the influence of permeation of NaF; NaF+oleic acid and IPA; control solution against the average concentration of NaF in PBS solution followed by post hoc test with LSD test. Search for normality and homogeneity of data performed before the test with Shapiro Wilk due to small sample size per group (n = 3). When the results of normality and homogeneity of the data showed the significance of ≥ 0.05, it could be proceeded with a parametric test that is one-way ANOVA test (Figure 1).

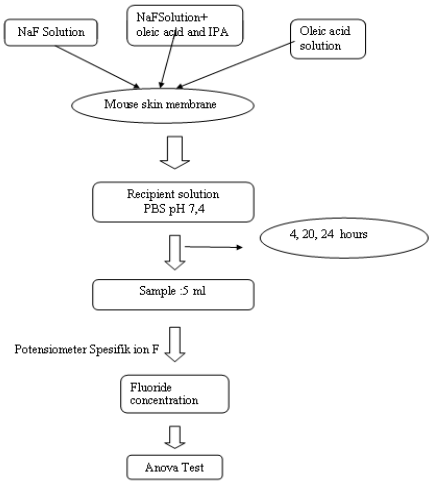

Figure 1. Research process

Ethical clearance was prepared before data collection as involved animal. The permission from Ethic Committee Faculty of Dentistry Gajah Mada University. Three white mice were prepared, then euthanasia was performed and the skin hair were removed with electric shaver and cut with mal punch (Figure 2 dan 3).

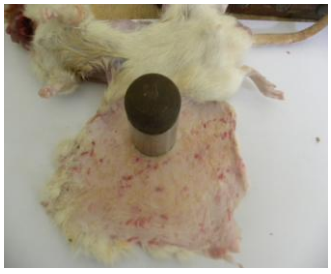

Figure 2. Mouse skin preparation

Figure 3. Donor solution preparation

After the mouse skin was ready, the next step was preparing donor and recipient solution for permeation test. Three test tubes were prepared and then the recipient compartment filled with 20 ml of PBS, mounted with mouse skin used a special clamp to the side of the outer skin (stratum corneum) faced the donor compartment (Figure 4).

Figure 4. Transport test using *Franz Like Difussion Cell*

The next step took 5 ml of sample after interval time of 4, 20, 24 hours from the neck cell (recipient compartment) using a special pipette volume. Subsequently filled again with PBS solution from the opposite side of the compartment using a pipette volume. Sample placed in a sealed plastic container and then stored until ready for fluoride concentration analysis to BATAN (Figure 5).

Figure 5. Sample taken from neck cell

RESULTS

The fluoride concentration increased in both of

Edisi Cetak Dentika Dental Journal, Juli 2012 (ISSN: 1693-671X)

the NaF solution and NaF + oleic acid and IPA solution, however in control group solution there was no increscent even though decreased gradually. In the first hour fluoride concentration in the NaF+ oleic acid and IPA solution is higher than the NaF solution group (0.34 and 0.09 ppm) as well as at subsequent time intervals and the highest difference at 24 hours when fluoride concentration in the NaF solution + oleic acid and IPA is 5,27 ppm and the NaF solution is 4,94 ppm. In the control group there was also a very small content of fluoride concentration (Table 1).

Table 1. Mean fluoride concentration (mg/L) after transport that of 4,20 and 24 hours on three groups solution

Solution	Duration time		
	4 hours (n=3)	20 hours (n=3)	24 hours (n=3)
NaF	0,09±0,05	0,30±0,01	4,94±0,01
NaF+oleic acid and IPA	0,34±0,05	4,95±0,16	5,27±0.01
Oleic acid (Control)	0,03±0,01	0,03±0,01	0,02±0,00

Statistical analysis showed the influence of transport time to fluoride concentration in the NaF solution group; NaF+ Oleic Acid and IPA solution and control solution. One-way ANOVA test showed that in the NaF solution have significant influence of transport time. The post Hoc test showed that transport time was significant at the 20 and 24 hours.

DISCUSSION

The results indicated the presence of NaF on skin permeability. Although the penetration was very small compared to the concentration of NaF was used as the donor solution but this has been proved that the presence of fluoride through mice skin. The concentration of NaF used as the donor solution is 1000 ppm while the highest fluoride concentration in recipient compartment is 5.27 ppm (in the NaF solution plus oleic acid and IPA after 24 hours). While the lowest concentration is 0.05 ppm in the NaF solution after 1 hour of transport test.

Very low permeability of NaF across the membrane skin according to[9] in which the epidermal layer of skin has the most robust outer structure that resembles a brick and hard to be penetrated by hydrophilic drugs. In theory, Sodium Fluoride that has high solubility in water can not penetrate to skin however it has a low molecular weight so these compounds can penetrate between the cells in the stratum corneum layer by passing through the gaps between cells.[15]

An effort required to speed up or increase the fluoride ion permeability. One of the ways is the addition of chemical enhancers.[16] This method is the most practical and relatively inexpensive compared with other methods. The use of chemical transport and increased the penetrating power of the drug has already been widely used, for example on research using oleic acid to increase penetrating power of bupranolol, the use of Propylene Glycol and Isopropyl Alcohol.[12]

The use of enhancers in this study is the consideration according to which states oleic acid is non irritating and biocompatible with fluoride and this chemical is easily obtained.[13] Oleic acid mechanism is by bringing fluoride to penetrate to the layer of lipid on the stratum corneum and interact with proteins between cells.[15] The combination of oleic acid and Iso Propyl Alcohol (IPA) as chemical enhancer also to increase transport of Papaverine hydrochloride and compared to other chemical enhancer this combination was significanly improving permeability of mice skin. The mechanism of action of various chemical permeation enhancers may be attributed to its activity on lipophilic matrix and/or hydrophilic protein gel in stratum corneum, which acted through interaction with intercellular lipids, led to disruption of its organization and increasing fluidity. Some of them may also interact with intercellular protein, keratin denaturation

In the control group fluoride concentration was still found, although very small concentrations of fluoride (0.03 ppm), this is likely due to the bias in the measurement of the concentration of fluoride with specific Potentiometer Fluoride.

Although the results in trials indicated the penetration of fluoride transdermally however fluoride presence was small and had not been able to achieve the desired dose so it needs to be tested again with the use of other enhancers or with trials in other ways such as basting hyper transport material on the skin surface. The result can also be used as an indicator to formulate a new fluoride modality via skin (trsndermally) such as patch. In conclusion, fluoride can permeate via mice skin and chemical enhancer can increase the permeation. From the research result some suggestions can be drawn: 1). an advance in vitro test with higher concentration of chemical enhancer needs to be conducted and 2). another method of using chemical enhancer by topiccaly applied to skin surface can be done as follow up research.

References

1. Featherstone JDB, Adair SM, Anderson MH, Berkowitz RJ, Bird WF, Crall JJ, et al. Caries management by risk assessment: Consensus statement. CDA J 2002; 31: 257-69.
2. Dawes C. What is the critical pH and why does a tooth dissolve in acid? J Can Dent Assoc 2003; 69: 722-24.
3. Marinho VCC, Higgins JP, Logan S, Sheiham A. Fluoride mouthrinses for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2003; (3): CD002279.
4. Ismail AI, Hasson H. Dental caries and fluorosis: a Systematic Review. J Am Dent Assoc 2009, 140 (Suppl 3): 278-9.
5. Ellwood R, Fejerskov O, Cury JA, Clarkson B. Fluoride in caries control. In: Fejerskov O, Kidd E, Dental caries: the disease and its clinical management. Munksgaard Blackwell; 2008: 287-323.
6. Featherstone JDB. Delivery challenges for fluoride, chlorhexidine and xylitol, BMC Oral Health 2006; 6 (Suppl. 1): 58-60.
7. Toumba KJ. Slow release devices for fluoride delivery to high-risk individuals, Caries Res 2001; 35 (Suppl. 1): 10-3.
8. Aggarwal G. Development, fabrication and evaluation of transdermal drug delivery system: A Review. Pharmainfo. 2009.
9. Subedi RK, Oh SY, Chun MK, Choi HK. Recent advances in transdermal drug delivery, Arch Pharm Res 2010; 33 (3): 339-51.
10. Chandra A, Sharma PK, Irchhiaya R. Effect of alcohol and enhancers on permeation enhancement of Ketorolac. Asian J Pharm 2009; 3: 37-42.
11. Anonymous. New hoodia patch overcomes absorption problems of slimming pills and capsules. http://www. iulren.com (Maret 2011).
12. Rowe RC, Sheskey PJ, Owen SC., Handbook of pharmaceutical excipient, 5th ed., Pharmaceutical Press, 2006: 624-27.
13. Prabhakara P, Koland M, Harish VK, Shankar G, Ahmed MG, Charyulu N, et al. Preparation and evaluation of transdermal patches of papaverine hydrochloride, J Res Pharm 2010; 1(3) 259-66.
14. Pathan IB, Setty M., Chemical penetration enhancer for transdermal drug delivery systems J Tropical Pharmaceutical Res 2009; 8 (2): 173-79.
15. Benson HAE. Transdermal drug delivery: penetration enhancement technique. Current Drug Deliver 2005; 2: 23-33.
16. Wang J, Ruan J, Zhang C, Yujie YE, Cai Y, Wu Y. Development and evaluation of the sinomenine transdermal patch, J Pharm Science 2008; 21 (4): 407-10.