In vitro study of fluoride absorption via mouse skin

(Studi penelitian in vitro absorpsi fluoride melalui kulit tikus)

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ABSTRACT

Background: Fluoride plays important roles in reducing dental caries by improving remineralization process and strengthen email through forming fluorapatite which is more resistant to acid. Systemic fluoride have disadvantages due to its difficulties to control the dose and related to first hepatic metabolism. Fluoride in systemic mode without passing metabolism with small and controlled dose need to be developed such as through Transdermal route. Purpose: The purpose of this research is to reveal the possibility how fluoride solution with and without enhancer can penetrate skin. Methods: Quasy experimental design with post test only control group design 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. Three groups of donor cell were fluoride solution; fluoride added oleic acid solution; oleic acid solution and recipient solution CMF PBS 0,1 M pH 7,4. Sample was taken for time interval of 1, 4, 8, 12, 24 hours and fluoride contained was measured by Potensiometer Spesific Ion Fluoride. Results: Research result showed an influence of transport test both on NaF solution and NaF+oleic acid solution (Anova Sig: 0.00 and 0.00) however on control group showed no signficancy (sig. 0.07). Solution of NaF+oleic acid had higher penetrating power than other solutions. Conclusion: It can be concluded that fluoride can penetrate to the skin and chemical enhancer addition can increase the penetration.

Key words: Fluoride, oleic acid, transport test, permeation

INTRODUCTION

Dental caries is a disease involves multi factorial aspects of dietary carbohydrates and bacteria in the oral cavity, such as Streptococcus mutans and Lactobacillus.1 A new theory of caries states the balance of minerals structure in enamel is determined by balancing of demineralization and remineralization process that occurs in oral environment. Each time sucrose attached biofilm, the bacteria will convert sugar into acid, increase solubility of enamel to acid.5

Among several caries preventive strategies, the use of fluoride has been proven t be effectively reduce the incidence of caries based on clinical trials, literature reviews and meta-analysis involving various fluoride preparations.3 Current research recommends the use of fluoride in low doses, administered continuously, slow release fluoride is more effective than the use of topical application.4 Toumba and Cursons states low-dose fluoride is slowly released to maintain the good bioavailability of the therapeutic window.5

Due to some shortcomings of the various preparations of fluoride, one should consider an alternative route that is more convenient for the patient in terms of application and can achieve the desired dose therapeutic.6 Transdermal drug delivery systems (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.7 The step
include diffusion through the lipophilic Stratum Corneum (SC), this is the most barrier 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. Molecular weights of drug should be less than 500 daltons, hidrophilic and lipophilic characteristic (log P: 1-3), melting point below 200° C and dose less than 10 mg/ day.8

Fluoride compounds commonly used in 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 met the high melting point (993° C) and lipid insoluble, but current technology can overcome these problems through modification of the media to penetrate the skin barrier,9 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. Several transport-enhancing ingredients such as Propylene Glycol (PG), Iso Propyl Alcohol (IPA) which is used by Chandra et al or d-limonene.10,11 According to Rowe et al.12 Oleic acid is a permeability-enhancing ingredient that is often used because of its low skin sensitivity and biocompatibility to hydrophilic drug. In vitro study about trial of fluoride solution proved that fluoride when added oleic acid can absorb through mouse skin faster,13, 14 however the study did not compare with oleic acid alone as control solution.

Based on the background above, the research problem are how is fluoride penetration to mouse skin when added with oleic acid compared to NaF without oleic acid and oleic acid as control.

**MATERIALS AND METHODS**

Quasy-Experimental design with Post Test Only Design approach was used. The study population is a 500 ml solution of phosphate buffer (calcium-magnesium 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 permeation test of time interval 1, 4, 8, 12, 24 hours. Dependent variable of this study was contact time of NaF, NaF+ oleic acid and oleic acid solution as control group into mouse skin on transport test while independent variable was amount of fluoride permeated.

Data collection started with donor solution preparation. Three donor solutions were prepared in this research: NaF solution, NaF + oleic acid solution and oleic acid solution as a control. Method of making NaF solution: NaF powder 300 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 10,000 ppm. Preparation NaF + oleic acid same as making a solution of NaF; 300 mg NaF powder added 2 ml aquabidest and 1 ml oleic acid. The third solution was a solution containing 3 ml oleic acid.

Next step was mouse skin preparation. Mouse was sacrificed, the skin was dissected using electric shaver; lipid tissue under the skin was cleansed, the skin tissue was cut using a mould that has been adjusted by means of cell transport. Skin tissue is stored in sealed plastic containers were given a solution of PBS, stored in a refrigerator until used. In vitro permeation study was implemented with modified Franz diffusion like cell were used for the release and permeation study of NaF solution. Donor compartment was sealed, membrane separation
between donor and recipient compartments was mouse skin with a diameter of 1.25 cm with 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 20 ml PBS pH 7.4 and continuously stirred using a magnetic bead with a speed of 50 rpm at room temperature.

At each predetermined time interval of 1, 4, 8, 12, 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 were used with time test transport factors to determine the influence of permeation of NaF ; NaF+oleic acid; and oleic acid against the average concentration of NaF in PBS solution.

It can be concluded that fluoride can penetrate to the skin and added chemical enhancer can increase the penetration. From the result some suggestions can be drawn: 1) an advance in vitro test with higher concentration of oleic acid need to be conducted to find out the mechanism of chemical enhancer; 2) another method of using oleic acid by topically applied the chemical to skin surface can be done as follow up research; 3) another research can be done using other kind of chemical enhancer such as IPA and d-limonene.

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