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Determination of Permeation Pathways of Clindamycin Phosphate into the Skin

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ABSTRACT

Extensive research has been conducted in the recent years with a focus on drug administration via the skin for both topical and systemic drug delivery. Understanding the drug permeation through the skin is crucial for the development of an optimal product. In this study, the permeation of clindamycin phosphate through the pig's ear was evaluated. Both plugged and non-plugged skin was used. In non-plugged skin, the hair follicle orifices were open and a significant amount of clindamycin phosphate was detected. However, plugged skin, in which hair follicle orifices were artificially blocked, clindamycin phosphate can only penetrate to a less extent through interfollicular epidermis and possible through sweat glands. The study was performed using a Franz-type diffusion cell for 24 hours. The samples were withdrawn for each time interval and were analyzed by UV spectrophotometer. Cumulative amount of permeated clindamycin phosphate was compared using the drug concentration. The difference in the percentage of drug permeated through plugged and non-plugged was57.67%. Based on the obtained results, it can be concluded that the follicular route is an important route for the drug delivery through the skin.

Keywords: Clindamycin phosphate, Follicular route, Acne vulgaris and Pig's ear skin.

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INTRODUCTION

Acne is an exclusively human disease and a unique condition of human sebaceous follicles of the face, chest and back that begins in the prepubertal child. Factors promoting the development of acne are increased sebum production, ductal cornification, bacterial colonization of the pilosebaceous ducts and inflammation[1]. Clindamycin is a lincosamide antibiotic. Clindamycin works primarily by binding to the 50s ribosomal subunit of bacteria. The effectiveness of topical clindamycin in preventing inflammatory lesions is based on its demonstrated *in-vivo* activity against *Propionibacterium acnes*, bacteria that are central to the pathogenesis of acne. In addition, clindamycin has direct anti inflammatory effects and is more lipophilic than some other antibiotics[2].Clindamycin completely suppressed the growth of *Corynebacterium acnes* organisms, whereas erythromycin and tetracycline will not depress the *C acnes* counts[3].

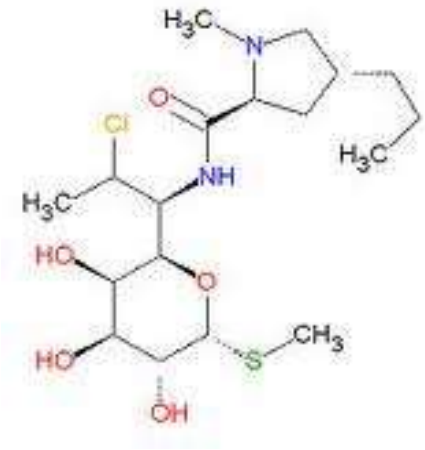

Figure 1: Structure of Clindamycin

Compounds can penetrate through the stratum corneum via three routes which are intracellular, transcellular and transappendageal. Once it has transferred through the epidermis, a compound may be carried away by the dermal blood circulation or to be transported to deeper tissue. The relative significance of these penetration pathways will be largely dependent on the physiochemical characteristics of the drug molecules, particularly the partition and diffusion coefficients into the protein or lipid regions[4]. Therefore, the objective of the present study was to confirm permeation pathway of Clindamyc in through follicular route. The further, aim was to compare drug concentration of Clindamyc in between plugged and non-plugged skin.

MATERIALS AND METHOD

Experimental animals for pig's ear skin ware purchased from pigs farm situated in Kuala Selangor, Selangor, Malaysia. The research study was performed by using two pig ears from freshly slaughtered pigs. Clindamycin phosphate 1% USP was used as a model penetrate for this study. It

was obtained from Pfizer Inc. (New York, USA). Nile red coloring agents was purchased from PPB Group Berhad, (Kuala Lumpur, Malaysia). Silicone grease was obtained from Synco Chemical Co. Ltd. (Bohemia, NY, USA). Potassium dihydrogen phosphate, Sodium Chloride, Disodium phosphate, Hydrochloric acid and Methanol were obtained from Sigma Aldrich Co. Ltd. (MO, USA). Eppendorf tubes were also obtained from Sigma Aldrich Co. Ltd. (MO, USA).

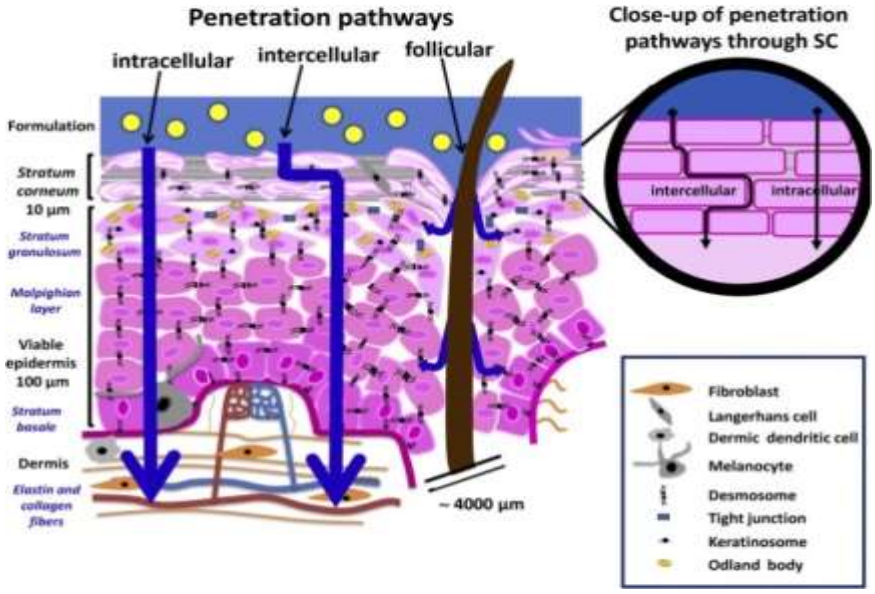

Figure 2: Skin Permeation Pathways[4]

Equipment's

Franz vertical glass diffusion cell was purchased from Permegear Inc. (Hellertown, USA). pH meter was purchased from Hanna Instrument Ltd. (Middleborough, MA, USA). Sonicator was obtained from Restek, (Bellefonte, PA,USA). Water bath was obtained from Brook Field Engineering Laboratories, Inc. (Middleborough, MA, USA). Analytical balance GR-200 was obtained from A&D Company Ltd. (Toshimaku, Tokyo, Japan). Magnifying glass was obtained from Lik Soon Sdn. Bhd. (Kuala Lumpur, Malaysia). Shaver series 500 were obtained from Philps Electronics N.V. (Kuala Lumpur, Malaysia). UV Spectrophotometer was obtained from Thermo Fischer Scientific, Inc.(Waltham, MA, USA).

Methods

The pig ears were cleaned carefully with distilled water and washed out of minerals for 2 hours before the experiments. The fat under the pig ear skin were removed and only the stratum corneum and the epidermis or dermis remains[5]. 0.2g of silicone grease was mixed with 0.25mg of nile red coloring agent to make plugging agent. Toothpick stick was used to plug the hair follicles in the pig ear skin by using the plugging agent. A circular piece of the pig ear skin was sandwiched securely between the two halves of the Franz diffusion cells with the stratum corneum side facing

the donor chamber. The receiver chamber was filled with 16mL of distilled water (pH7.0±0.2) which was continuously stirred by small magnetic stirrer and thermo-state at 37±2°C throughout the experiment. The receptor was always continuously stirred with a magnetic stirrer at 480 rpm and thermostated at 32±0.5 °C with circulating jacket[5,6]. 1mL of the Clindamycin solution was put in the donor chambers applying on the pig ear skin. The donor chambers were covered with a paraffin film[5]. The receptor cells were filled with 0.1 M phosphate buffer (pH 7.4). Next, the samples were loaded in the donor chamber at time 0 and then withdraw from the receiver chamber at 0, 0.5, 1 to 8 and 12 hours. After sampling, the same volumes of fresh phosphate buffer were added to the receptor cell to keep the volume constant. The concentration of clindamycin solution permeated in the both hair follicles of plugged and non-plugged skin were determined by using UV Spectrophotometer[5]. The wavelength that was used for the analysis was 210nm. A standard curve was plotted and the concentration drug permeation was determined by using the standard curve. Lastly, cumulative amount of permeation drug for both plugged and non plugged skin versus time were plotted and skin permeation decreasing ratio was determined.

RESULTS AND DISSCUSSION

Standard Curve for Clindamycin

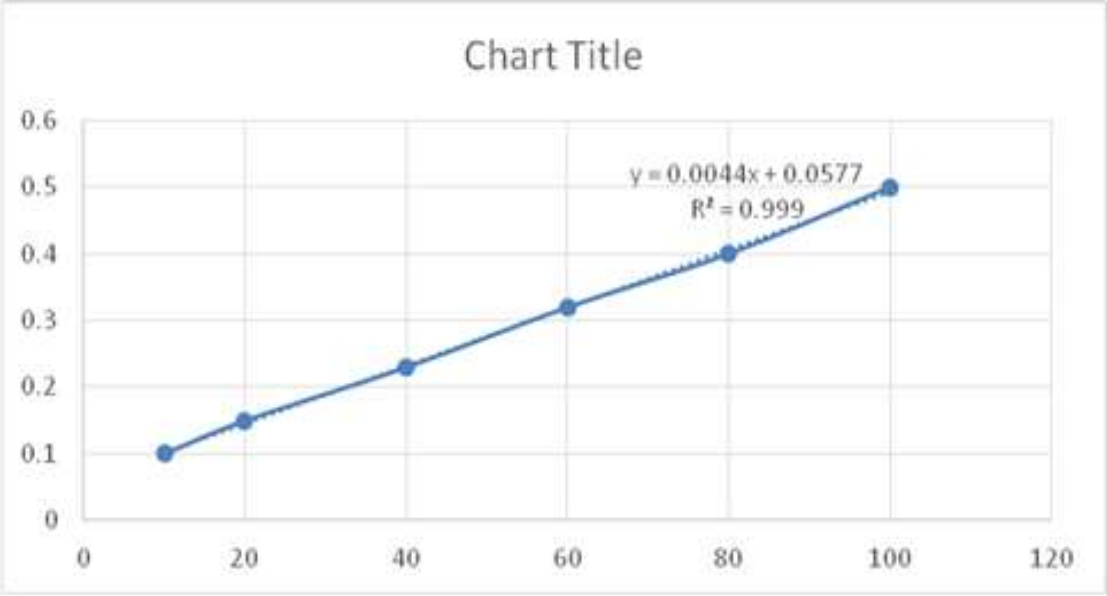

Figure 3: Standard calibration curve for Clindamycin Phosphate. The graph was plotted according to the known concentration derived from serial dilution of Clindamycin Phosphate.

From the graph, concentration of drug permeation in plugged and non-plugged skin was calculated.

Concentration of Clindamycin permeation in Non-plugged Skin

Table 1 Concentration of drug permeation in non-plugged skin

Time (h)	Conc. (µg/mL)
0	0
0.5	80.00
1.0	101.00
1.5	125.30
2.0	129.30
2.5	148.00
3.0	145.30
3.5	156.30
4.0	126.50
4.5	165.30
5.0	166.50
5.5	170.20
6.0	174.40
6.5	169.50
7.0	158.60
7.5	164.90
8.0	165.00
18.0	168.20
18.5	157.20
19.0	147.80
19.5	143.20
20.0	151.10
20.5	170.20
21.0	170.00
21.5	169.80
22.0	167.20
22.5	167.20
23.0	175.70
23.5	175.70
24.0	145.20

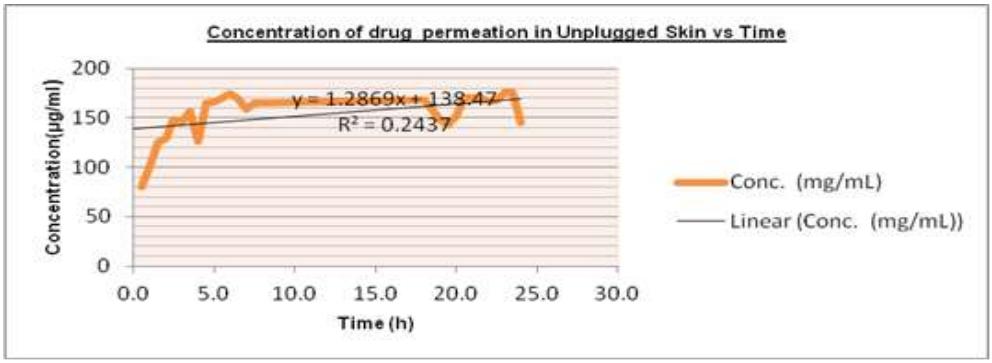

Figure 4: Concentration over time graph for Clindamycin phosphate drug permeation into the non-plugged pig ear skin.

Concentration of Clindamycin Phosphate Permeation in Plugged Skin

Table 2 Concentration of drug permeation in non-plugged skin

Time (h)	Conc. (µg/mL)
0	0
0.5	43.00
1.0	7.00
1.5	10.30
2.0	38.00
2.5	58.90
3.0	51.00
3.5	39.30
4.0	37.20
4.5	41.00
5.0	23.00
5.5	20.00
6.0	40.80
6.5	11.10
7.0	5.80
7.5	48.00
8.0	52.80
18.0	50.20
18.5	23.00
19.0	48.30
19.5	48.20
20.0	7.90
20.5	42.50
21.0	49.60
21.5	39.30
22.0	47.00
22.5	13.60
23.0	43.60
23.5	41.30
24.0	41.30

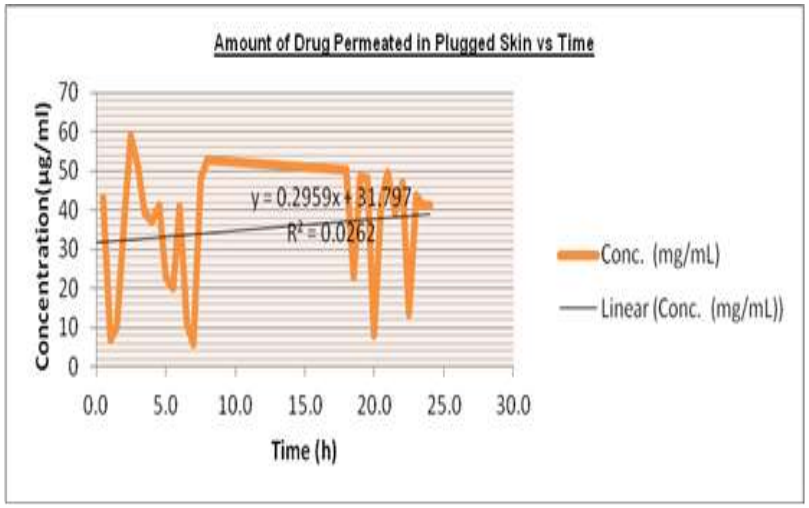

Figure 5: Concentration over time graph for Clindamycin phosphate drug permeation into the plugged pig ear skin

Cumulative amount of Permeation Drug for Plugged and Non- Plugged Skin

Table 3 Cumulative amount of permeation of Clindamycin Phosphate for Pluggedand non-plugged pig ear skin

Time (h)	Cumulative amount of permeation drug/Available permeation area (µg/cm^2)	
	Plugged skin	Non-Plugged skin
0.0	0	0
0.5	136.05	253.11
1.0	46.44	364.75
1.5	60.84	498.69
2.0	154.29	582.14
2.5	241.89	714.35
3.0	250.17	789.42
3.5	241.97	906.32
4.0	257.53	900.34
4.5	290.56	1094.56
5.0	256.78	1191.75
5.5	260.28	1297.53
6.0	337.39	1406.97
6.5	266.47	1490.00
7.0	255.98	1551.28
7.5	392.77	1660.81
8.0	435.07	1754.29
18.0	456.68	1857.64
18.5	422.03	2016.40
19.0	492.02	1976.94
19.5	518.99	2045.89
20.0	418.72	2151.79
20.5	532.66	2297.58
21.0	579.13	2393.11

21.5	574.56	2488.52
22.0	621.13	2576.23
22.5	542.01	2670.69
23.0	644.61	2792.05
23.5	661.97	2891.31
24.0	685.30	2894.08

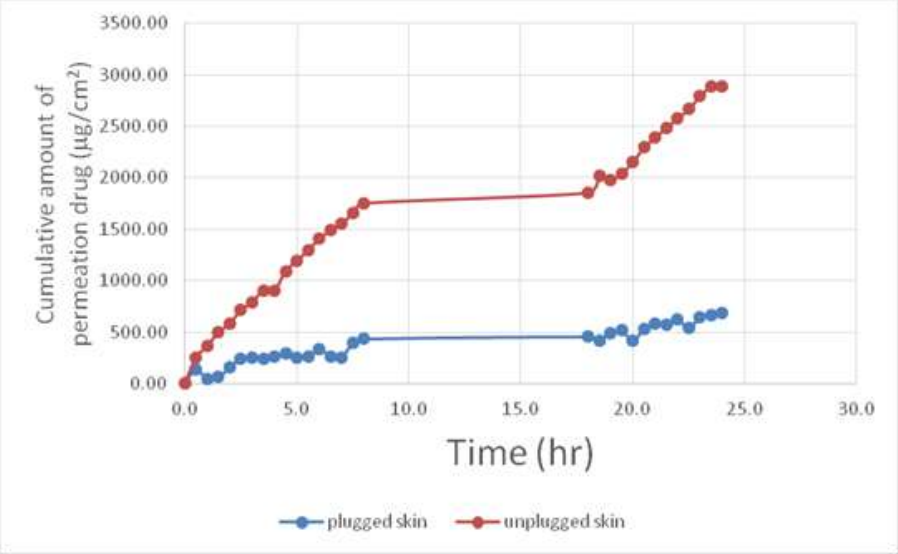

Figure 6: Graph of cumulative amount of permeation of Clindamycin Phosphate for plugged and non-plugged skin versus time. Based on the results, the concentration of clindamycin Phosphate permeation in non-plugged skin was higher compared to plugged skin.

The results are significant differences (P< 0.05) between the concentration of plugged and non-plugged skin.

Skin permeation Decreasing Ratio Calculations

$$\frac{\text{(P values in HF non-plugged skin – P values in HF plugged skin)}}{\text{P values in HF non-plugged skin}} \times 100 = 57.67\ \%$$

Clindamycin phosphate is the water soluble ester of clindamycin and phosphoric acid. It is a minimally active pro-drug that is rapidly hydrolyzed *in-vivo* to the active compound, which is clindamycin base, a bacteriostatic antimicrobial. In the treatment of acne vulgaris the target organism is primarily the Propionibacterium acnes in the skin, although other skin pathogens may be present[7]. Presence of these pathogens is associated with inflammatory lesions in the skin which have been related to direct stimulation of the innate immune response through activation of Toll-like receptor-2 and other chemo tactic factors. Topical antimicrobial treatments deliver local “skin” doses of antibiotic well in excess of the MIC of P. acnes. Comedonal concentrations of clindamycin following atopical application of 1% solutions averaged 597 µg/g of Comedonal

material with systemic absorption of <0.5 ng/mL plasma concentration. In this research, we used pig skin as an alternative to human skin to determine the concentration of drug permeation. Using histological and echogenic techniques, pig skin was reported to be similar to human skin. Using a series of compound, it was shown that the skin of pig ear has the closest permeability characteristics to that of human skin. In vitro studies of with human skin have also been shown to correlate well with in vivo studies with pig's skin. Pig ear skin has also been reported to be a good model for investigating delivery of topically applied formulations into hair follicle. The experiments to determine the concentration of drug permeation in both plugged and non-plugged pig ear skin was carried out by using Franz Diffusion cells. The Franz Cell apparatus consists of two primary chambers separated by a membrane. In our experiments, pig ear skin was used as the membrane. The Clindamycin solution is applied to the skin via the top chamber. The bottom chamber contains fluid from which samples are taken at regular intervals for analysis. This testing determines the amount of drug that has permeated the membrane at each time point. The chamber is maintained at a constant temperature of 37˚C. For the analysis of concentration of drug permeation, we have used UV Spectrophotometer. Ultraviolet-visible Spectrophotometry refersto absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The wavelength that was used for determination of Clindamycin Phosphate concentration is 210nm. A standard curve was plotted and the concentration of drug permeation for both plugged and non-plugged skin was determined according to the standard curve (Figure 3). By using Excel spreadsheet, cumulative amount of permeation drug for plugged and non-plugged skin has been calculated and a graph that compares both of the amounts has been plotted. According to the graph of cumulative amount of permeation drug versus time non-plugged skin shows highest amount of drug permeation compares to plugged skin (Figure 6). Skin permeation decreasing ratio also has been calculated for plugged and non-plugged skin and it shows 57.67%. Besides that, T test result also showed that the two tailed P value is less than 0.0001. This difference is considered to be extremely significant and thus proves that the amount of drug permeation in non-plugged pig ear skin is higher compared to plugged skin. Based on the results that we have obtained throughout the research, we can conclude that the concentration of drug permeation in non-plugged pig ear skin is definitely higher compared to plugged pig ear skin. Thus, this proves that the major permeation pathway for Clindamycin is via hair follicular route. There are few studies that have been performed with different types of compound to support the theory of drug permeation. In Otberg studied in 2007, she stated about the role of hair follicles in the percutaneous absorption of caffeine. Faster absorption of caffeine was obtained when the follicle orifices were open. The

results revealed that the caffeine were detectable within 5 minutes when the hair follicle orifices remain open[7]. When the follicular orifices were closed, penetration of caffeine into the skin took longer time which was 20 minutes and lower blood caffeine concentration was measured. This study shows that follicular route is highly significant for drug permeation compared to intercellular route[8].

CONCLUSION

The pilosebaceous unit plays an important role in permeation and penetration processes of topically applied compounds. The human hair follicle is not only an important reservoir but also an entry point for topically applied substances and significantly contributes to the transport of drugs into the skin. In our research, the concentration of Clindamycin permeation in non-plugged pig ear skin is higher compared to plugged pig ear skin. Our aim and objective has been achieved. Through this research, we have proven that the major permeation pathway for Clindamycin into the skin is via hair follicular route. Thus, more studies on follicular permeation have to be carried out to emphasize the importance of hair follicles as diffusion pathways and to optimize the effect of topical antibiotics in treating acne. The limitation that have encountered while performing this research is the analysis instrumentation. The result for this study can be improved by using surface ionization mass Spectrophotometry (SI/MS) technique. This instrumentation technique is able to show the clear differences between the penetration pathways of drugs[8].

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