International Journal of Cosmetic Science, 2007, **29**, 277–282

In vitro human nail penetration and kinetics of panthenol

X. Hui*, S. B. Hornby†, R. C. Wester*, S. Barbadillo*, Y. Appa† and H. Maibach*

*Dermatology, University of California San Francisco, Surge 110, Box 0989, 90 Medical Center Way, San Francisco, CA 94143 and †Neutrogena Corp., 5760 West 96ht Street, R&D Building, Los Angeles, CA 90045, U.S.A.

Received 3 November 2006, Accepted 28 January 2007

Keywords: human nail, *in vitro* transungual delivery, kinetics, non-lacquer film formulation, penthanol

Synopsis

The *in vitro* absorption of panthenol into and through the human nail was examined in this study. Panthenol, the alcohol form of pantothenic acid (vitamin B5), is believed to act as a humectant and improve the flexibility and strength of nails. A liquid nail treatment formulated with panthenol (2%) was compared to a solution of panthenol (2%) in water. Fingernail specimens were dosed daily for 7 days with either the nail treatment (non-lacquer film forming) formulation or aqueous solution with sampling performed every 24 h. Panthenol concentrations were determined in the dorsal surface, interior (by drilling and removal) and in the supporting bed under the human nail. Panthenol levels in the dorsal nail ($R^2 = 0.87$; $P < 0.001$), nail interior ($R^2 = 0.94$; $P < 0.001$) and nail supporting bed ($R^2 = 0.79$; $P < 0.003$) showed a significant linear increase with each day of dosing. Significantly more panthenol was delivered into the interior nail and supporting bed by a nail treatment formulation than from an aqueous solution. The film acts not only as a reservoir of panthenol, but also acts to increase the hydration of the nail and the thermodynamic activity of panthenol as well, thereby enhancing diffusion.

Correspondence: Dr Xiaoying Hui, Department of Dermatology, University of California San Francisco, Surge 110, Box 0989, 90 Medical Center Way, San Francisco, CA 94143, U.S.A. Tel.: +(415) 502 7761; fax: (415) 753 5304; e-mail: huix@derm.ucsf.edu

Introduction

Panthenol, the alcohol form of pantothenic acid (vitamin B5), was selected for study because it is believed to increase the water storage capacity of the nails and improve their flexibility and stability [1]. However, information about quantitative transungual delivery of panthenol in nail formulations into/through human nail plate has been lacking. This study compares the formulation effect of non-lacquer film forming nail care formulation and aqueous solution on the penetration of [^{14}C]-panthenol into the deeper layer of the human nail, and to determine the flux rate and kinetics of [^{14}C]-panthenol from the nail treatment using an *in vitro* nail diffusion system [1]. The data may help to understand permeation character and rate of panthenol in each layer of the human nail and to design an optimum transungual delivery system.

Materials

[1-^{14}C]-Panthenol was obtained from American Radiolabeled Chemicals, Inc. (St Louis, MO, U.S.A.). The radiochemical purity and specific activity of the chemical were determined by the manufacturer as 99% by HPLC and 50 mCi mmol^{-1}, respectively. Non-radiolabelled DL-panthenol and nail formulation base were obtained from Neutrogena Corporation (Los Angeles, CA, U.S.A.). This base contains ethanol, acrylates copolymer and phytantriol.

Healthy human fingernail plates were collected from adult human cadavers and stored in a closed container at 0–4°C. Before the experiment, the

nail plates were gently washed with normal saline to remove any contamination, then re-hydrated by placing them for 3 h on a cloth wetted with normal saline.

Methods

Penetration study

Two formulations, 2% [^{14}C]-panthenol in 98% nail treatment base and 2% [^{14}C]-panthenol in water were tested for penetration of panthenol into human nail plate. Aliquots of 15 μL of the nail treatment and aqueous formulations containing 0.07 ± 0.01 and 0.08 ± 0.00 μCi radioactivity, respectively, were applied to human nail plate once per day for 7 days with a daily washing before dosing.

Details of the nail incubation procedure have been given previously published by Hui et al. [2, 3]. Briefly, a healthy nail plate was mounted in a one-chamber diffusion cell (Permegear, Inc., Hellertown, PA, U.S.A.) with the dorsal nail surface (top centre) open to the air and the ventral surface making contact with a small cotton ball acting as a nail supporting bed. The supporting cotton ball under the nail was wetted by normal saline providing moisture for the nail plate, and the degree of hydration was monitored and controlled during the experiment. The incubation period started 24 h prior to the first dose, and ended 24 h after the final dose. Aliquots of 15 μL of the test [^{14}C]-panthenol formulations, containing approximate 0.07–0.11 μCi radioactivity, were applied to the dorsal surface of the nail plate with an HPLC microsyringe once daily with approximately 24 h between applications for up to 7 days. Starting on the second day, each morning before dosing, the surface of the nail was washed with cotton tips in a cycle, as follows: two times with ethanol, then with 50% Ivory® liquid soap (Procter & Gamble, Cincinnati, OH, U.S.A.), then twice with distilled water. The washing samples from each cycle were pooled and the radioactivity was measured. After completion of the dosing and the incubation phase, the nail plate was transferred to a cutting holder (Fig. 1) for sampling. Under the controlled humidity and temperature, we did not observe any abnormal nail plate colour change, hydration changes or any fungal growth during the 7-day dosing period. The nail plate was secured in position so that the outer dorsal, dosed surface faced the holder. The cutting holder was moved to bring the plate surface just barely in contact with the cutter tip. The drill was then turned on and a fine adjustment moved the stage towards the cutter tip, removing a powder sample from the nail. In this way, a hole approximately 0.3–0.4 mm in depth and 7.9 mm in diameter was drilled in each nail, enabling the harvest of powder sample from selected areas across the nail plate. The dorsal/intermediate centre of the nail, which was the immediate area of dosing, will be referred to as the dorsal nail in this paper. The powdered nail sample drilled from the centre of the inner surface facing the nail bed, approximately 0.3–0.5 mm deep and beneath the dosed surface of the nail plate, but not including the dorsal surface, the ventral/intermediate nail plate centre, will be called the interior nail.

The nail outside the dosing area (and also the sampling area) was cut away and saved as the

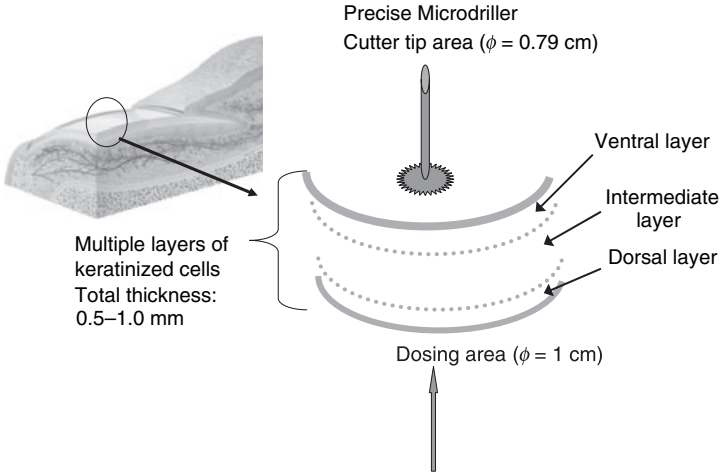

Figure 1 Schematic of the precise microdrilling technique for assessing depth of penetration

'remaining nail plate'. All the nail plate samples were individually collected into glass scintillation vials and weighed. The weights of the powdered nail specimens that were collected from each of the measurements were the same ($P > 0.05$). The nail samples were dissolved and then the amount of [^{14}C]-panthenol was measured with the scintillation counter. In addition, the amount of [^{14}C]-panthenol remaining in the liquids used to wash the nail plate each day, the ring that secured the nail plate into the diffusion cell to prevent leakage, and the liquids used to rinse inside of the diffusion cell were combined and assayed as described above. To ensure that all of the [^{14}C]-panthenol was accounted for, the mass balance of total radioactivity recovery of [^{14}C]-panthenol measured from surface washing, nail samples and supporting bed cotton ball samples after the 7-day dosing period was calculated. It was found that the total recovery was 104.4 ± 9.6% from the specimens of the non-lacquer brush on nail conditioner and 93.52 ± 11.75% from the aqueous group demonstrating that there was no loss of [^{14}C]-panthenol from the *in vitro* nail diffusion system.

Kinetic study

Seven groups of three nail plates each were formed. Each nail plate in each of the groups received 15 μL of the 2% [^{14}C]-panthenol nail treatment formulation containing 0.11 μCi radioactivity once daily for up to 7 days using the technique described above. Every 24-h period after the dose, samples of each group were collected and daily penetration rate and flux were determined following the sequence diagrammed in Table I.

Table I Sequence of dosing and sampling the nail plate specimens in the [^{14}C]-panthenol kinetic study

	Dosing day: D							
Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Group 1	3	9						
Group 2	3	3	9					
Group 3	3	3	3	9				
Group 4	3	3	3	3	9			
Group 5	3	3	3	3	3	9		
Group 6	3	3	3	3	3	3	9	
Group 7	3	3	3	3	3	3	3	9
	Sampling day: S							

Results

Table II shows the thickness of whole nail plate, the depth of the ventral surface core sample removed by cutter, the percentage of the whole nail thickness, and the actual weight of powdered nail sample collected for both the aqueous solution and the nail care formulation-treated nails. There were no statistical differences between the groups ($P > 0.05$).

Table III gives the panthenol equivalents in the nail and supporting bed cotton ball in nail conditioner group and in the aqueous group normalized by the weight of the extracted nail material. After 7 days of topical dosing, the amount of panthenol in the interior nail samples (ventral/intermediate) and in the supporting bed cotton ball in the nail formulation group was significantly higher than that in the aqueous group ($P < 0.05$). Correspondingly, the amount of panthenol on the dosed dorsal surface of the nail treated with the nail formulation was 34% lower than that of the aqueous panthenol solution.

The reproducibility of the *in vitro* nail diffusion system was demonstrated by comparing the results obtained after 7 dosing days in the penetration experiment to those obtained after the 7th day in the kinetic experiment (Table IV). There was no statistical difference found between any of the values.

Table V gives the daily flux rate of panthenol penetrating into/through the nail plate in the lacquer group. Steady-state fluxes of panthenol were reached within 24 h and lasted over the dosing period.

Discussion

After 7 days of topical dosing, it was observed that [^{14}C]-panthenol concentration in the interior nail plate in the nail treatment group was as high as twofold as that of the aqueous group ($P < 0.05$) (Fig. 2). Triple the amount of [^{14}C]-panthenol was observed in the supporting bed cotton ball of the nail in the nail treatment group compared with that in the aqueous group ($P < 0.05$).

Human nail has been shown to behave like a hydrophilic gel membrane [4] in that the flux of a substance across the nail layers is dependent upon the water solubility of the substance as well as the permeability of the nail plate. The diffusion rate may be low for molecules with poor water solubility.

Table II Attributes of the nail core sampled from the interior of the nail plate

Treatment	Nail thickness (mm)*	Core sample depth (mm)	Percentage of nail plate represented by the core*	Weight of powdered nail sample collected (mg)*
Nail treatment	0.69 (0.17)	0.31 (0.08)	45.06 (6.81)	6.08 (2.47)
Aqueous solution	0.83 (0.09)	0.37 (0.08)	45.33 (8.20)	5.39 (1.38)

Numbers in parentheses are the standard deviations. *Non-significant ($P > 0.05$).

Table III [^{14}C]-Panthenol in nail and supporting bed samples after 7-day treatment

	Mean [^{14}C]-panthenol equivalent (mg eq. g^{-1} nail), $n = 5$		
	Nail treatment	Aqueous	Significance (P)
Dorsal	10.05 (3.52)	13.51 (6.16)	>0.05
Interior	4.97 (0.90)	2.09 (1.99)	<0.05
Supporting bed cotton ball	0.80 (0.33)	0.28 (0.15)	<0.05

Numbers in parentheses are the standard deviations

Panthenol is hydrophilic ($C \log P = -1.75$) with a relatively low molecular weight (MW = 205.2) [1]. Thus, it has reasonable water solubility and a small size which are expected to be contributing factors for its observed ability to penetrate across the layers of the nail plate. It is also likely that the enhancement of the penetration of panthenol from the nail treatment vs. the aqueous solution may be due to the fact that the nail treatment solvent quickly evaporates after application leaving a concentrated film on the dorsal surface of the nail plate. The film acts not only as a reservoir of panthenol, but also acts to increase the hydration of the nail and the thermodynamic activity of the panthenol, thereby enhancing diffusion.

Table IV The measured panthenol from the various areas of the nail plate in the penetration and kinetic experiments after 7 days of dosing

	[^{14}C]-panthenol equivalent (mg eq. g^{-1} nail) after 7 dosing days*	
	Penetration study	Kinetic study
Dorsal	10.05	10.83
Interior	4.97	3.57
Supporting bed cotton ball	0.80	0.78

*Non-significant ($P > 0.05$).

Kinetics

It was found that the flux of panthenol through the nail plate reached a steady state within 24 h (Table V). The average flux in the interior nail plate was 10.25 ± 2.75 g equivalent panthenol cm^{-2} h^{-1} and in the nail supporting cotton bed (completely through the nail) was 1.47 ± 0.79 g cm^{-2} h^{-1}. The cumulative panthenol concentration in and through the nail layers increased linearly with time (Fig. 3) and can be mathematically predicted as follows:

Dorsal Nail: Panthenol Conc. (mg eq. g^{-1}) = −0.20 + (0.054*hours), Slope of the line = 0.054 ($R^2 = 0.94$, $P < 0.001$);

Interior Nail: Panthenol Conc. (mg eq. g^{-1}) = −0.29 + (0.021*hours), Slope of the line = 0.021 ($R^2 = 0.87$, $P < 0.001$);

In nail supporting bed cotton ball: Panthenol Conc. (mg eq. g^{-1}) = −0.07 + (0.004*hours), Slope of the line = 0.004 ($R^2 = 0.79$, $P = 0.003$).

It has been suggested that the main barrier to drug permeation in the human nail plate may be

Table V Flux of panthenol into/through the human nail plate from the nail treatment

	Flux (μg equivalent panthenol cm^{-2} h^{-1})							
	0–24 h	0–48 h	0–72 h	0–96 h	0–120 h	0–144 h	0–168 h	Average
Interior (nail core)	12.02 (2.62)	8.24 (0.45)	7.18 (1.72)	8.29 (0.76)	13.16 (0.40)	12.60 (1.84)	13.85 (4.85)	10.25 (2.76)
Supporting bed cotton ball	0.54 (0.20)	2.26 (0.93)	1.55 (0.63)	1.41 (0.25)	1.22 (0.24)	1.82 (0.70)	3.03 (1.19)	1.47 (0.79)

Each value represents the mean (SD) of three samples.

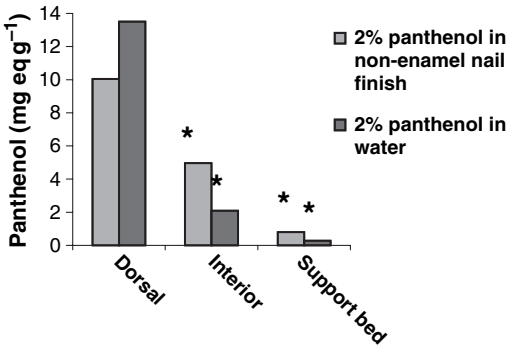

Figure 2 The amount of panthenol measured in the various layers of the nail plate was higher from the nail treatment than that from the aqueous solution. *The differences observed are statistically significant ($P < 0.5$).

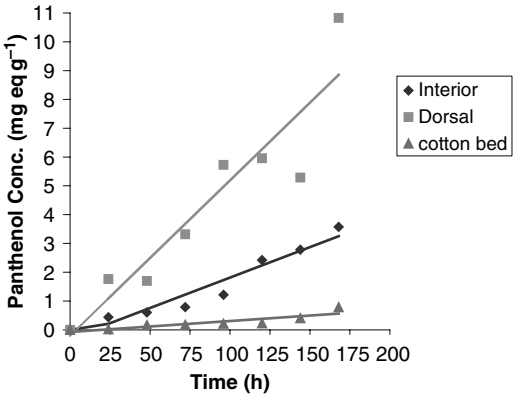

Figure 3 Panthenol concentration as a function of time.

the low diffusivity of drugs in the dorsal layer [5]. As can be seen in Fig. 3, the slope of the panthenol concentration vs. time curve of the dorsal layer is higher than that of the interior layer, which was higher than that for the supporting bed cotton ball. This shows that the concentration over time of panthenol was the highest at the dorsal layer suggesting that the affinity of panthenol to the dorsal nail plate was high, therefore, a factor in the relatively low diffusivity compared to that at the deeper layers of the nail plate.

The variation in the diffusion rate of panthenol as a function of location within the nail plate is similar to the diffusion behaviour of 5-fluorouracil, another small water-soluble molecule into human nail plate, which was explored in an *in vitro* study conducted by Kobayashei et al. [5]. They concluded that the drug permeation characteristics of the layers of the human nail plate are as follows: the dorsal layer is characterized by low diffusivity to substances; the intermediate layer is characterized by low lipophilicity, and higher diffusion rates of hydrophilic molecules; and the ventral layer is characterized by high lipophilicity and diffusion rates that can be lower than those of the intermediate layer. These researchers found that the diffusion rates of their probe molecules were faster in the interior of the nail plate than the dorsal or ventral layers. Our observed panthenol kinetic data are consistent with their findings.

Conclusion

The nail treatment formulation shows better effectiveness on enhancement of transungual panthenol delivery into/through the deeper layer of the human nail plate than the aqueous solution of panthenol. Taken together, the panthenol penetration data provide the basis for examining vehicle effects on the delivery of actives into human nail, and may stimulate investigation into enhanced activity in nail formulations.

Acknowledgements

This study was funded by Neutrogena Inc. and the University of California.

References

1. Hui, X., Wester, R.C., Barbadillo, S. and Maibach, H.I. Nail penetration: enhancement of topical delivery of antifungal drugs by chemical modification of the human nail. In: *Textbook of Cosmetic Dermatology* (Baran, R. and Maibach, H.I., eds), pp. 57–64. Taylor & Francis, London and New York (2005).
2. Hui, X., Chan, T.C.K., Barbadillo, S., Lee, C., Maibach, H.I. and Wester, R.C. Enhanced econazole penetration into human nail by 2-*n*-nonyl-1, 3-dioxolane. *J. Pharmaceut. Sci.* **92**, 142–148 (2003).
3. Hui, X., Wester, R.C., Barbadillo, S. *et al.* Ciclopirox delivery into the human nail plate. *J. Pharmaceut. Sci.* **93**, 2545–2548 (2004).
4. Walters, K.A., Flynn, G.L. and Marvel, J.R. Physico-chemical characterization of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. *J. Pharm. Pharmacol.* **35**, 28–33 (1983).
5. Kobayashi, Y., Miyamoto, M., Sugibayashi, K. and Morimoto, Y. Drug permeation through the three layers of the human nail plate. *J. Pharm. Pharmacol.* **51**, 271–278 (1999).