Characterization of Skin Permeation of Vitamin C: Theoretical Analysis of Penetration Profiles and Differential Scanning Calorimetry Study

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A mechanism for the relatively high permeability of vitamin C in relation to the change in the protein domain of the stratum corneum has been proposed. Firstly, the skin permeation characteristics of vitamin C (*l*-[1-^{14}C]-ascorbic acid) using whole skin and stripped skin of the hairless mouse were investigated. By employing a double layer model, physicochemical properties such as diffusivity and solubility of vitamin C in each skin layer, stratum corneum and viable skin were determined. Then, the high skin permeation rate of vitamin C was characterized. A differential scanning calorimetry, (DSC), study was employed to investigate the effect of vitamin C on the stratum corneum, a major diffusion barrier for the skin transport of the compound.

Vitamin C was found to permeate rapidly through the skin, in spite of its low lipophilicity. The diffusivity determined from the lag-time was approximately 1000 times higher in the stripped skin, compared with whole skin. There is a dramatic increase (10-fold) in the permeation rate in stripped skin indicating the major barrier presented by the stratum corneum to the skin permeation of vitamin C.

The DSC profile showed four very distinctive transitions near 100, 128, 135 and 145 °C which are associated with protein transitions. Comparing normal skin, the peaks are sharpened and there are additional phase transitions above 90 °C. An increase in sharpness reflects an increase in the hydration state of the sample, as hydrogen bonds between H_2O molecules and other hydrogen donating chemicals of skin components become major chemical bonds in hydrated samples. The higher permeation rate of vitamin C observed may be due to its enhancing effect on the hydration capacity of skin and solubilizing action on the protein domain of the stratum corneum.

Key words skin permeation ; vitamin C; ascorbic acid; stratum corneum; enhancer; differential scanning calorimetry

Vitamin C (*l*-ascorbic acid: $C_6H_8O_6$, M.W. = 176.1) has been used in skin care products for several decades, with claims of various benefits such as antiaging,[2] moisturizing[3,4] and skin whitening effects.[5,6] Vitamin C is a six carbon compound structually related to glucose and other hexoses. Although vitamin C is a very hydrophilic compound with a partition coefficient between octanol and water of 0.02 ± 0.002, the skin permeation of vitamin C has been confirmed by several scientists. In 1937, Kasahara and Kawashima[7] reported that water soluble vitamin C can be absorbed through the human skin, as shown by the increase of vitamin C in mother's milk following the application of a 30% solution of ascorbic acid to mammalian skin. In 1958, Takeuchi[8] studied the skin absorption of vitamin C by measuring the decrease in radioactivity of [^{14}C]-vitamin C after applying [^{14}C]-vitamin C topically. The skin areas with less sweat glands and hair follicles, permitted less permeation of ascorbic acid. After removing the stratum corneum, there is a remarkable increase in skin permeation. In 1967, Imai *et al.*[9] reported that after topical application of vitamin C in vitamin C-deficient animals, the alkaline phosphatase concentration in blood and vitamin C concentration in liver proved that vitamin C was absorbed through the skin.

In 1989, a report described the skin permeation properties of vitamin C such as its metabolism and the effect of endogeneous tissue distribution of vitamin C on its skin permeation profile. Tojo and Lee[10] reported the initial "bursting" in the skin permeation profiles of vitamin C using HPLC. Vitamin C is an essential vitamin for humans and the tissue vitamin C distributes endogeneously diffuses into the receptor solution during the transient period of penetration. By comparing the simulated penetration profiles with the experimental ones, assuming homogeneous distribution for tissue vitamin C, the initial tissue concentration of vitamin C in the skin was evaluated (Cinitial: 2.7 μmol/ml). They also investigated the metabolism of vitamin C in the skin and found that vitamin C is hardly metabolized.[11] Although there are several reports about the high skin permeation of vitamin C, there is no quantitative analysis of skin permeation characteristics and we still do not know how this very hydrophilic compound which has four hydroxyl groups can affect the hydration capacity of the stratum corneum and the skin enhancing mechanism by altering the composition of the stratum corneum.

It is known that a lipophilic compound can penetrate the stratum corneum more easily than a hydrophilic one. Initially it was thought that vitamin C would have difficulty penetrating the skin since it is very hydrophilic. However we observed a higher skin permeation compared with other hydrophilic drugs, and so investigated how this hydrophilic drug can penetrate the skin very easily. The permeation properties of vitamin C, its physico-chemical parameters such as diffusivity, partition coefficient and solubility in the stratum corneum and viable skin were evaluated by the double-layer model.[12] The effect of vitamin C on the stratum corneum was also studied by differential scanning calorimetry (DSC).

In this report, a theoretical analysis of the penetration profiles of vitamin C and the thermal profiles of the DSC study are discussed.

Experimental

Vitamin C was obtained from Sigma Chemical Co., (St. Louis, MO). Radio-labeled vitamin C (*l*-[1-^{14}C]-ascorbic acid, 10.0 Ci/mmol) was

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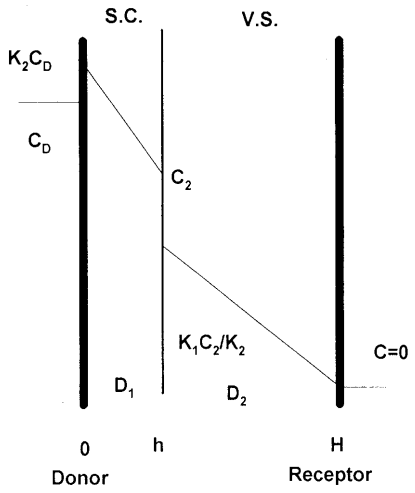

Fig. 1. Concentration Profile of Drug through Skin Based on a Bi-Layer Model

C_D: Drug concentration in donor compartment (mg/ml). K_1: Partition coefficient of drug between skin and donor solution. K_2: Partition coefficient of drug between stratum corneum and donor solution. D_1: Drug diffusivity through viable skin (cm^2/sec). D_2: Drug diffusivity through stratum corneum (cm^2/sec).

purchased from E.I. Dupont NEN Research (Boston, MA). Bioflour (E.I.Dupont NEN Research, Boston, MA) was used as a liquid scintillation cocktail. A liquid scintillation counter (Rack Beta 1214-001, LKB Instruments Inc., Gaithersburg, MD) was used to count the total radioactivity of the labelled compound. A differential scanning calorimeter (Delta Series DSC7; TAC7/3 Instrument controller;graphics plotter, Perkin Elmer) was used to investigate the effect of vitamin C on the thermal transitions of the stratum corneum of hairless mouse skin.

Permeation Study The permeation study was performed as reported previously.[10] In brief, freshly excised hairless mouse skin was mounted between the half cells of the *in vitro* skin permeation system. The volume of each cell was 3.5 ml and the diffusion area was 0.64 cm^2. Either intact skin or stripped skin was used. Fifty percent glycerin aqueous solution was selected as a donor and a receptor solution based on stability studies of vitamin C in various stabilizing solutions such as propylene glycol and polyethylene glycol (PEG) 400.[13,14] The effect of glycerin in donor and receptor solutions upon the skin permeation of drugs has been investigated.[15] The experimental results show that glycerin in donor solution did not change the skin permeation properties such as steady state skin permeation rate and lag-time. So, 12 mg/ml vitamin C in 50% glycerin solution and 50% glycerin solution were used as donor and receptor solutions respectively. During the entire period of the penetration experiment, the receptor solution was maintained under sink conditions.

At predetermined time intervals, 30 μl receptor solution was withdrawn, mixed with scintillation cocktail, counted for several minutes and assayed for the drug concentration with a liquid scintillation counter.

Data Treatment Since vitamin C is hardly metabolized in skin, the skin permeation study with radio-tracer analysis represents the permeation properties of vitamin C alone. So, the total radioactivity of ^{14}C-vitamin C detected in the receptor solution as a function of time was considered to be the total amount of drug passing through the skin. The permeation rate was evaluated from the slope of the linear portion of the profile, and the lag-time was defined as the time-axis intercept.[12] The membrane diffusivity was determined from the time lag method.[16] Following an initial lag-time of 6 h, the amount of vitamin C appearing in the receiver side was linear with time for the duration of the experiment (24—48 h). From a linear least squares regression analysis of these data, the amount of vitamin C appearing in the receptor side as a function of time was determined. This value, when divided by the specific activity of vitamin C in the donor solution (9.8×10^5 disintegration per minute, DPM) and the area of exposed skin, yielded the flux (μg/cm^2/h). The partition coefficient and solubility of drug in each skin layer, stratum corneum and viable epidermis, were determined using a bi-layer skin model.[12] The concentration profile of drug through skin based on the bi-layer model is shown in Figure 1. We used 10 μm and 370 μm as the thickness of the stratum corneum and the viable dermis, respectively.

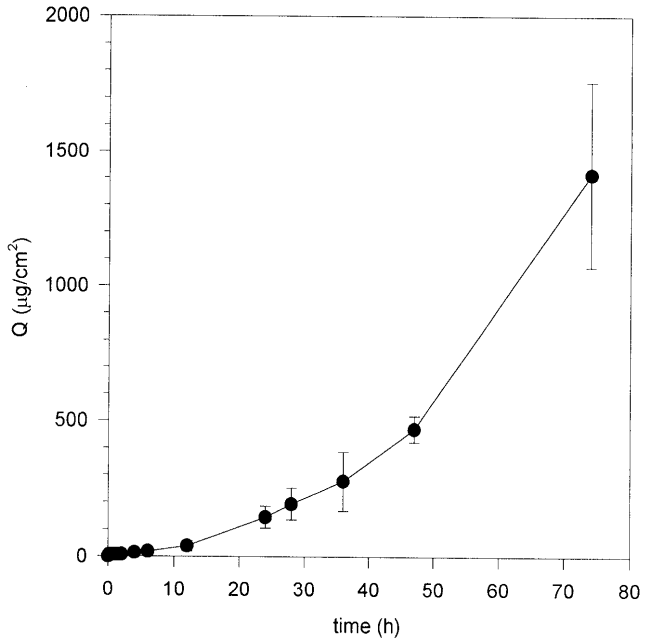

Fig. 2. Skin Permeation of Vitamin C (1-^{14}C-*l*-Ascorbic Acid) through Whole Skin

Donor solution; 13 mg/ml Vt.C in 50% glycerin solution. Receptor solution; 50% glycerin solution

DSC Study Skin equilibrated with drug solution was mounted on filter paper, wetted with 0.5% (pH 7.4 phosphate buffer) trypsin solution in a covered petri dish for 14 h at room temperature. Since the hydration state of the sample affects the DSC thermal profile, the experimental conditions to separate the stratum corneum were controlled with regard to equilibration time and drying conditions. The sratum corneum layer was carefully separated from viable skin. The samples were washed three times with distilled water and dried with "kim-wipes". A clear and thin layer of stratum corneum was then put into the sample cell for the DSC study. The weight of each individual stratum corneum sample in the DSC study was 17$\pm$2 mg. The temperature was increased from 30 to 170 °C at a rate of 5 °C per minute. The DSC study was repeated 5 times since the profile of thermal transitions can be influenced by the water content of the sample and the sample preparation procedure. The DSC thermal profile of vitamin C treated skin was reproducible.

Results and Discussion

Skin Permeation of Vitamin C across Whole and Stripped Skin Figures 2 and 3 show the skin permeation of vitamin C across whole skin and stripped skin of hairless mouse. The permeation rates through the whole skin (3.43$\pm$0.74 μg/cm^2/h) and stripped skin (33.2$\pm$5.2 μg/cm^2/h) were determined. It has been proposed that the poor permeability of the stratum corneum of the skin is due to an ordered matrix of intercellular lipid and to the low water content.[17] Due to the lipophilic nature of the stratum corneum, a hydrophilic compound has difficulty in permeating the stratum corneum. Tojo *et al.*, studied the steady-state rate of skin permeation of progesterone and its hydroxy derivatives across intact and stripped skin.[12] They found the steady-state rate of permeation and the solubility of progesterone and its hydroxy derivatives in the stratum corneum decreased gradually as the hydrophilicity of the penetrant increased. However, very hydrophilic vitamin C shows a short lag-time and high skin permeation rate when we compare the skin permeation rate of vitamin C with that of vitamin E (whole skin; 1.58$\pm$0.58 μg/cm^2/h and stripped skin;

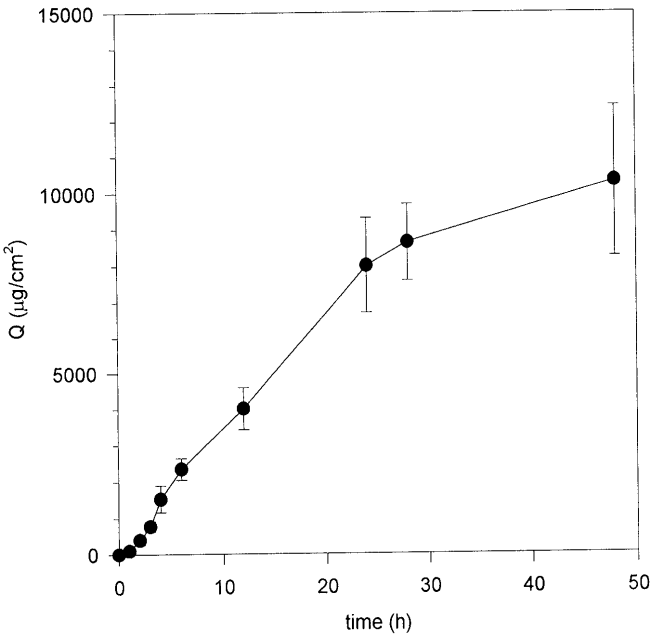

Fig. 3. Skin Permeation of Vitamin C through Stripped Skin

Table 1. Physico-Chemical Properties of Vitamin C

Molecular weight		176.12
Partition coefficient (Octanol/Water)		0.02±0.002
Donor solution (13 mg/ml)		6.2% of Cs
dQ/dt ($\mu g/cm^2/h$)	Whole skin	3.43±0.74
	Stripped skin	33.2±5.2
D (cm^2/s)	Stratum corneum	$4.6\pm0.95\ (\times10^{-11})$
	Viable skin	$6.34\pm0.79\ (\times10^{-8})$
Solubility (mg/cm^3)	Stratum corneum	21.34±3.93
	Viable skin	56.0±7.39
	Whole skin	56.5±15.5
Partition coefficient (stratum corneum/viable skin)		0.25±0.13

$3.34\pm1.68\ \mu g/cm^2/h$).[11] Also, hydrophilic vitamin C has a higher permeation rate and marked higher solubility in the stratum corneum, compared with hydrocortisone and six other compounds.[12]

Physico-Chemical Properties of Vitamin C Table 1 summarizes the theoretical parameters of skin penetration of vitamin C. The diffusivity of vitamin C through the stratum corneum and the viable skin are similiar to other drugs.[18,19] Vitamin C shows a 4-fold increase in the partition coefficient in viable skin. However, the solubility of vitamin C in the stratum corneum (21.34± 3.93 mg/ml) is about 40 times higher than that of vitamin E (0.49±0.20 mg/ml).[13] The higher solubility of vitamin C in the lipophilic stratum corneum may imply some kind of interaction between vitamin C and the components of stratum corneum.

DSC Study of Normal Skin Figure 4 is a thermal profile of the stratum corneum isolated from normal hairless mouse skin and prepared by 0.5% trypsin treatment for 14 h. The DSC thermal profile of normal skin showed a broad endothermic change from 50 to 150 °C. After

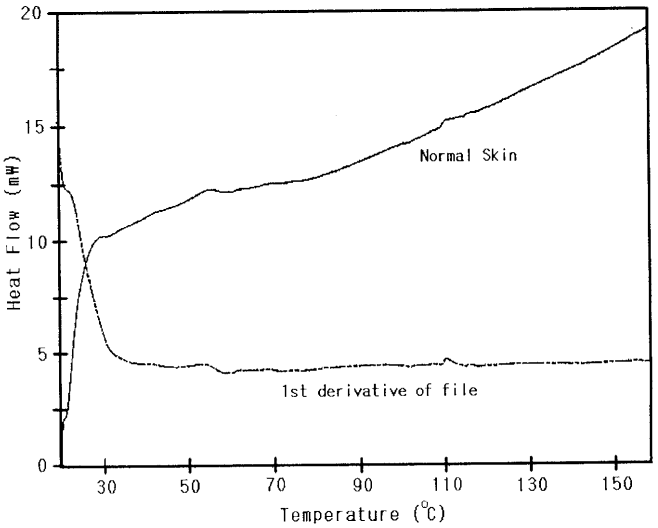

Fig. 4. DSC Thermal Profile of Stratum Corneum of Normal Skin

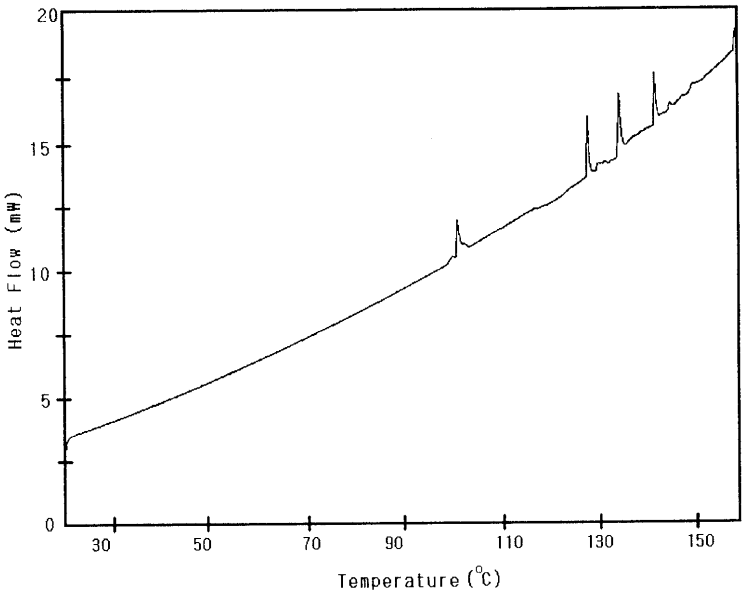

Fig. 5. DSC Thermal Profile of Vitamin C-Treated Stratum Corneum

investigating the DSC thermal profile of extracted lipid and protein from the stratum corneum, Potts[20] reported that the transitions near 60 °C is associated with lipids, whereas the peaks near 100 °C are associated with proteins.

DSC Study of Vitamin C-Treated Skin Figure 5 is a thermal profile of stratum corneum pretreated with 13 mg/ml vitamin C for 24 h. Compared with normal skin, the peaks are sharper and there are additional protein phase transitions above 90 °C. The profile shows four very distinctive transitions near 100, 128, 135 and 145 °C which are associated with protein transitions. Van Duzee[21] explained that the peak at 107 °C was due to denaturation of the keratinocyte envelop protein. Bulgin and Vinson[22] reported that the peak near 135 °C might be due to keratin denaturation. In highly hydrated samples, free water produces a peak near 100 °C whereas bound water is thought to be responsible for transitions near 115 and 135 °C. Several studies showed that the peak sharpened with increasing water content. Inoue *et al.*,[23] studied the effect of total water content on DSC thermograms. As the water content increased from 30 to 100%, a small endothermic peak becomes very distinctive and sharp. Our DSC thermal profile of vitamin C-treated stratum corneum showed sharpened phase transitions near 100, 128, 135 and 145 °C which support our point: Vitamin C has a higher skin permeation rate and this may be due to the skin hydration effect of vitamin C. As previously reported by several authors, an increase in sharpness reflects an increase in hydration state of the sample.[23] Hydrogen bonds between H_2O molecules and other hydrogen-donating chemicals of skin components become major chemical bonds in hydrated samples. An increase in sharpness reflects a thermal transition starting from a more homogeneous state.

Discussion

It has been reported that a major constituent of the stratum corneum is protein, and 90% is water-insoluble protein,keratin.[22] Nishihata *et al.*,[24] clearly demonstrated that the penetration rate constant of diclofenac in the presence of 25 mM vitamin C was approximately 10 times greater than that found in the absence of vitamin C. The penetration rate constant of diclofenac linearily increased as the vitamin C concentration increased in the donor solution. The protein thiol content in the stripped skin linearily increased as the vitamin C concentration increased. They postulated that vitamin C cleaves the disulfide bridges (S=S double bond) of keratin by reducing the S=S double bond into a SH–SH bond and increases the hydration capacity of the stratum corneum. Sun and Green[25] also reported that 2-mercaptoethanol, a strong reducing agent, cleaves disulfide bonds, causing enhanced hydration and further solubilization of proteins in keratinized tissues. Several studies[26,27] strongly support the idea that vitamin C enhances the hydration of the stratum corneum, as observed in the DSC thermal profile of vitamin C-treated stratum corneum.

The present experimental results of our DSC thermal profile suggest that vitamin C may change the protein domain of the stratum corneum. A very high permeation rate for vitamin C is observed in the skin permeation study, in spite of its hydrophilicity. The above explanation may involve alteration of the protein domain by vitamin C.

Conclusion

Vitamin C was observed to permeate rapidly through the skin and showed a higher solubility and diffusivity in the stratum corneum. A mechanism for the relatively high permeability of vitamin C in relation to the change in the protein domain of the stratum corneum has been proposed. The thermal profile changes seen following treatment with vitamin C suggest that vitamin C increases the hydration of the stratum corneum and changes its protein domain.

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References and Notes

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