Monitoring the Penetration Enhancer Dimethyl Sulfoxide in Human Stratum Corneum *in Vivo* by Confocal Raman Spectroscopy

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INTRODUCTION

The stratum corneum (SC) barrier typically consists of layers of corneocytes embedded in a lipid continuum that regulates barrier function. The lipid domain containing ceramides, cholesterol, and free fatty acids provides the major pathway for most drugs permeating across SC (1).

Penetration enhancers diminish the SC barrier function. The classic enhancer is dimethyl sulfoxide (DMSO) (2). Its mechanisms of action remain unclear, although DMSO disrupts lipid organisation and may displace protein-bound water (3).

Here we use confocal Raman spectroscopy to probe molecular interactions between a finite (depleting) dose of DMSO and SC, as functions of depth and time, providing novel information about residence time and location of DMSO in human SC *in vivo*.

MATERIALS AND METHOD

Instrumentation

A confocal Raman microspectrometer, described elsewhere (4–6), acquired data with a depth resolution (for planes) of 5 μm (6). A palm was positioned on an aluminium stage containing a CaF_2 window; this served as a reference plate to determine skin surface position and prevent movement artefacts. Spectra were calibrated and corrected for the instrument response (7).

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Methods

Raman spectra from a male and female were recorded from the ball of palm (30 s with 100 mW laser power on the skin). Sites were briefly cleaned with 97% ethanol.

Spectra from untreated SC were collected, then 4 μL of 80% v/v DMSO in a 5% v/v propylene glycol: water solution was placed within a template. After ambient drying, the template was removed and the skin surface was rinsed with pure water. A fresh template located the marked application site during spectral collection. The first spectra were obtained approximately 15 min after DMSO application. Next day the area was briefly cleaned and spectra were collected from the palm surface inwards in 10 μm increments to 120 μm.

RESULTS AND DISCUSSION

When investigating skin distribution of chemicals, it is necessary to establish which tissue layer (SC or viable epidermis, [VE]) is sampled. Thus, depth profiles from untreated SC identified indicators for when the laser no longer probed the SC but sampled the VE.

Depth Profile of Untreated Palmar SC

Fig. 1 presents spectra from within untreated palmer SC (not scaled but offset for clarity). Intensity differences with depth are notable; the skin surface (0 μm) provides a much lower intensity than from 10 μm, since at the surface only half of the measuring volume probes skin and the remainder locates in the CaF_2 window. Remaining spectra decrease in intensity as the laser focuses deeper into the skin, partly due to elastic light scattering that strengthens with increasing light path. The spectrum 120 μm below the surface is markedly less intense compared with that at 80 μm, consistent with the scatter coefficient being higher in VE than in SC. Another factor affecting signal intensity is increased water concentration in lower regions of the SC effectively reducing the signal contribution of lipids and proteins (6,7).

Spectral intensity decreases may indicate that VE is assessed instead of denser SC, but overall signal intensity does not designate clearly the skin layers. Chemically based markers were therefore used to distinguish between SC and VE. Natural moisturizing factor (NMF) is a mixture of amino acids, their derivatives, and salts produced in the lower SC. The concentration of these constituents changes markedly just above the SC/stratum granulosum boundary (5).

We determined NMF-to-protein signal ratios (5) as a function of palm skin depth to identify spectral markers useful for distinguishing between SC and VE (Fig. 2). NMF concentration drops rapidly between about 60 and 80 μm below the surface. Because NMF is in the SC only, this implies that palm SC has a thickness of about 80 μm. NMF vibrations at 1415 cm^{-1} (mainly from glycine, serine, and alanine) and 885 cm^{-1} (from pyrrolidone-5-carboxylic acid) are usually well resolved with *in vivo* Raman spectra of SC. For example (Fig. 1, arrowed), these vibrations are clear down to 50 μm, but disappear between 80 and 120 μm, indicating that here NMF is absent and SC is no longer sampled. Thus, NMF presence can be verified from vibrations at 1415 and 885 cm^{-1}, and hence these provide convenient markers to distinguish between SC and VE. However, we have determined NMF con-

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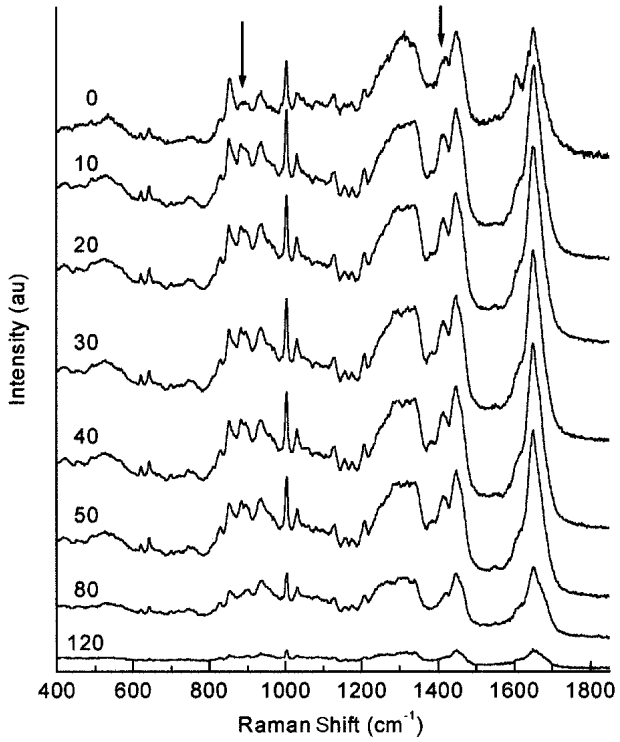

Fig. 1. *In vivo* Raman depth profile of untreated palm (0–120 μm). Spectra are to scale and offset along intensity axis for clarity. The arrows indicate some vibrational modes originating from Natural Moisturizing Factor.

centration profiles to distinguish unambiguously between skin layers.

Depth Profile of Palmar SC Treated with DMSO

80% v/v DMSO in 5% v/v PG/water (application mixture) possesses distinctive characteristic bands, notably a strong doublet at 671 and 702 cm^{-1}, assigned to symmetric and asymmetric ν(CSC) modes, respectively, and a broad 1030/1048 cm^{-1} band assigned to $\nu(S=O)$ stretching.

Fig. 3 illustrates data of DMSO in palmar SC collected 15 to 20 min after application. Compared with application mixture, DMSO in SC shows major shifts in several vibration positions; the $\nu(S=O)$ feature shifts to 1017 cm^{-1}, and the 671/702 cm^{-1} ν(CSC) doublet presents at 677/711 cm^{-1}.

DMSO complexes with water through dipole-dipole and hydrogen bonding interactions that are stronger than those formed between water molecules (8). DMSO also associates with polar portions of lipids and replaces water molecules as hydrogen bond acceptor, thereby associating with the N-H and C=O protein moieties. Significant, reproducible wavenumber shifts (Fig. 3) indicate DMSO interaction with one or more of these skin components.

Raman microscopic depth profiling studied DMSO-treated palm SC over 72 h. The DMSO vibrations dominated spectra obtained 15–20 min and 3½ h after treatment and were strongest at the palm surface and to 30 μm deep. After 72 h, the only DMSO vibration discerned was the very strong ν(CSC) mode at 677 cm^{-1}, appearing as a relatively weak signal in SC. These depth profiles indicated that some of the DMSO remained in SC for at least up to 72 h.

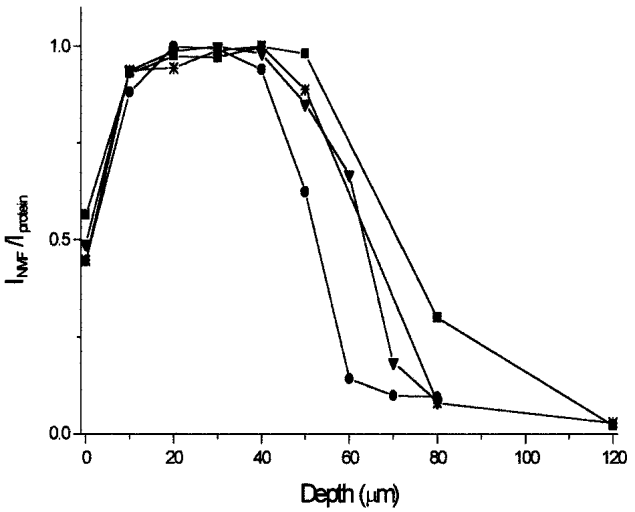

Fig. 2. Concentration profiles of natural moisturising factor (NMF) in palmer stratum corneum, normalized to maximum concentration. The left-hand ordinate represents ratio between the Raman signal intensity of NMF and of keratin.

To examine the distribution of DMSO through SC *in vivo* with time, we measured areas of $\delta(CH_2)$ vibration at 1450 cm^{-1} (integrated signal intensity of 1430–1470 cm^{-1} interval) from SC proteins and the ν(CSC) band of DMSO at 677 cm^{-1} (integrated signal intensity of 661–691 cm^{-1} interval). Area ratios of the DMSO/protein bands (A_{677}/A_{1450}) were plotted against skin depth (Fig. 4a to f). Fig. 4a presents ratios for depth profiles obtained 15 to 20 (squares), 31 to 36 (circles), and 39 to 45 min (triangles) after DMSO application. The first data set demonstrates that DMSO distributed throughout SC and that a small fraction may be present in VE; 120 μm NMF vibrations were absent, confirming that this spectrum was from VE.

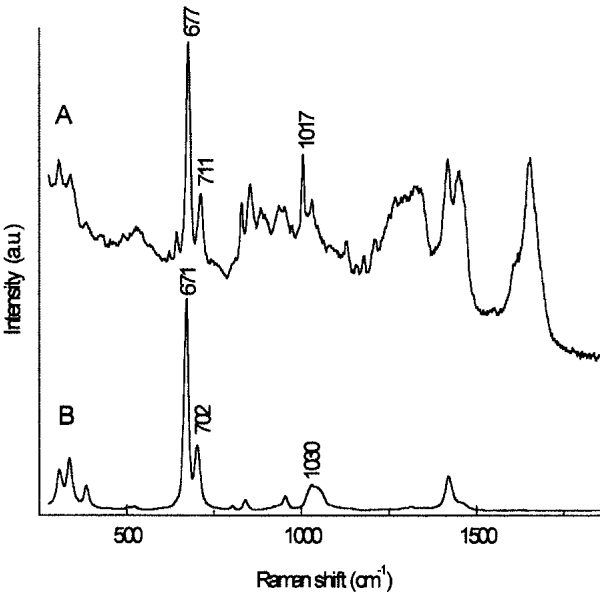

Fig. 3. A: Raman spectrum of palmar stratum corneum treated with dimethyl sulfoxide (DMSO), 17 min after application. Recording depth is 20 μm below the skin surface. B: Raman spectrum of DMSO application mixture (80% v/v DMSO in 5% v/v PG/water).

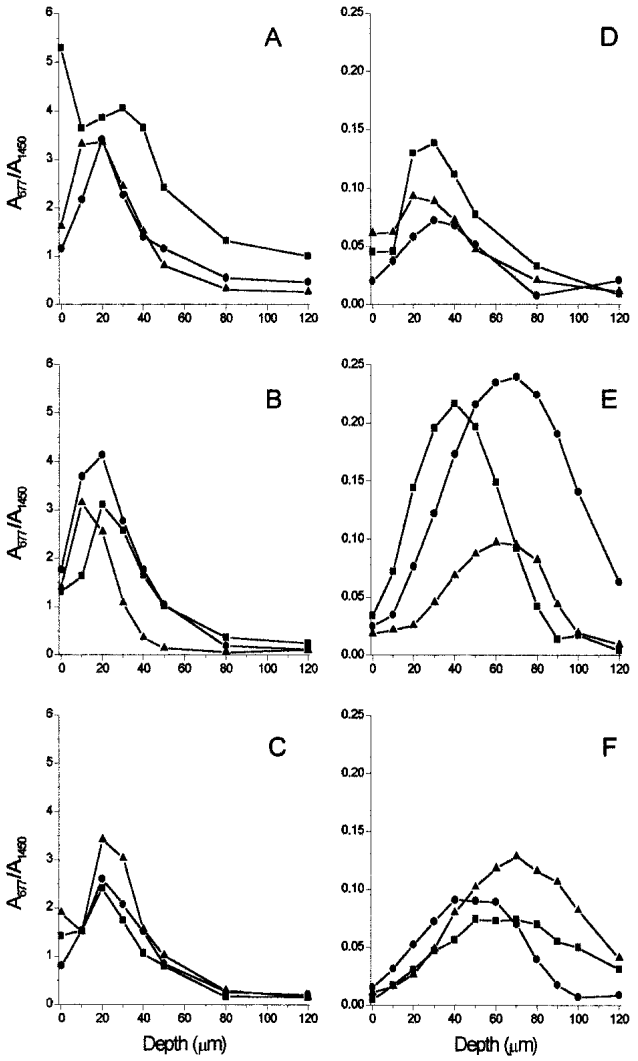

Fig. 4. Dimethyl sulfoxide (DMSO) distribution throughout palmar stratum corneum. Ratio of DMSO/protein band areas (A_{677}/A_{1450}) vs. depth. A: 15–45 min. B: 150–170 min. C: ~4 h D: ~22 h. E: ~48 hours. F: ~72 h postapplication.

Subsequent data, collected 30 to 45 min after dosing, showed different DMSO distributions. The DMSO/protein ratio had decreased significantly, i.e., there was considerably less DMSO present. In contrast to the earliest data, the enhancer was now largely confined to the outer 10 to 20 μm with very little detected at deeper layers or in VE.

Data collected up to 4 h post application (Fig. 4a to c) showed gradual permeation of DMSO through SC. Intensity of the DMSO peak relative to that of the endogenous protein remained essentially invariant, but it was evident that the sulfoxide was detected at increasing depths with time.

At 22 h postapplication (Fig. 4d) enhancer intensity relative to the protein modes dramatically decreased. This reduced intensity element of the enhancer progressed to increasing depths within SC with time; there were still detectable levels of DMSO within the tissue up to 72 h postdose (Fig. 4f).

One interpretation of Fig. 4 is that three mechanisms operate during DMSO permeation; initial rapid shunt route permeation (i.e., via hair follicles and/or sweat ducts) followed by pseudo steady-state transport across the bulk of the skin with the minor 72 h fraction attributed to highly bound enhancer. However, the spectral data do not support such a scheme. Firstly, the laser-sampling diameter is approximately 2 μm, thus it is unlikely that spectra will be recorded reproducibly from DMSO in a follicle or a sweat duct. Secondly, a very small dose of DMSO was applied to the skin (4 μL of 80% DMSO solution) and hence steady-state conditions would not arise. Finally, the positions of the vibrational modes from DMSO in the SC were invariant; no evidence was present that the residual 72 h fraction was any more bound than that detected 15 min after dosing.

Thus, it seems likely that Fig. 4 profiles arise simply from percutaneous absorption of a finite dose. Although clearly the enhancer interacts with SC components (binding), most small polar molecules traverse the tissue rapidly whereas a tail remains that permeates over an extended time.

Recently, FT-Raman spectroscopy probed DMSO effects on hydrated SC following 1-h treatment *in vitro* (9); DMSO progressively modified keratin from an α-helical to a β-pleated sheet conformation. SC lipids were also affected; at concentrations enhancing drug flux, DMSO modified lipids from the predominant all *trans* gel phase to a *trans-gauche* liquid crystalline phase. Here, the *in vivo* Raman spectra of DMSO-treated SC obtained were examined for changes that might indicate DMSO interaction with SC lipids and/or a conformational change of keratin. DMSO amounts applied were very small and there was no compelling evidence that it modified SC components. However, the full-width at half-height value of the amide I band for the surface spectrum in Fig. 3 was 92 cm^{-1}, markedly different to 58 ± 14 cm^{-1} for spectra of untreated SC. This measurement reflects changes in protein molecular environment, which together with a positional shift of this amide I band, implies that DMSO molecules may hydrogen bond directly to the protein C = O and N-H moieties.

CONCLUSIONS

Permeation of DMSO in human skin was studied *in vivo* using confocal Raman microspectroscopy. DMSO distribution in SC was monitored with depth and time. As expected, most of the dose permeated through SC within 20 min. Surprisingly, some remained within the tissue for hours and a small fraction was detected for days, decreasing steadily with time. Differences in DMSO vibrational modes provided evidence for interactions with water and possibly other polar tissue moieties. Changes in the amide I region were recorded from the skin surface, but firm conclusions regarding conformational changes of keratin due to DMSO interaction could not be drawn.

This study demonstrated a unique capability to monitor, *in vivo* and noninvasively, molecular permeation through human skin. Such investigations have major implications for design and evaluation of topical and transdermal formulations and for clinical evaluation of many therapeutic agents.

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