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An *in vivo* confocal Raman spectroscopic investigation of salicylic acid penetration: Variation with formulation parameters

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Abstract

Salicylic acid (SA) is widely used in leave-on antiacne formulations, typically at a 2% level. As a β-hydroxy acid, it is a milder active ingredient than either α-hydroxy acids or benzoyl peroxide. SA is a keratolytic agent, a bacteriocide, and a comedolytic agent. For these reasons, improving the efficiency of SA delivery is of interest. The objective of this work is to measure *in vivo* SA penetration from topically applied 2% SA leave-on products and to understand the penetration in terms of formulation parameters. Penetration of SA was measured in three depth zones—0–3 μm, 3–6 μm, and 6–9 μm below the surface—using *in vivo* confocal Raman spectroscopy. The delivery of SA from an emulsion, pH 4.0, and a hydrogel, pH 3.75, was compared and contrasted. A comparison of depth profiles reveals, e.g., significant differences in SA distribution between-treatment profiles at various time points after treatment, particularly 3–6 μm below the surface. The hydrogel exhibited the higher normalized level of SA in the 3–6 μm depth zone. Confocal in vivo Raman spectroscopy is proving to be a valuable tool in determination of details of penetration of products into the skin. The penetration of various 2% SA anti-acne product forms will be compared and contrasted in this presentation. Delivery of SA will be discussed in terms of formulation parameters such as phase, pH, and specific ingredients and molecular-level interactions.

INTRODUCTION

Salicylic acid (SA) is a widely used active ingredient in antiacne products. It serves several functions—as a keratolytic agent, as a comedolytic agent, and as a bacteriocide. It is a β-hydroxy acid and is milder than α-hydroxy acids or benzoyl peroxide. Nevertheless, it can be irritating at a pH of 3–4, the pH range at which SA is most efficacious in treatment of skin disease (1). One tactic for decreasing the irritation potential of SA is to control its delivery into the skin.

The delivery of SA into the skin can be affected by formulation parameters (2). A major difference in the formulae tested here is the amount of ethanol and the matrix containing SA. Ethanol is known to be a skin penetration enhancer and is used in many transdermal formulae and patches. It can accomplish this function by several means. Ethanol may act as a solvent for the active of interest. In addition, it can aid in optimizing the solubility

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of actives in the stratum corneum. Specific interactions between SA and the matrix may also be important.

MATERIALS AND METHODS

Volunteers signed informed consent before participation.

All formulae tested contained 2% SA. Products tested included a treatment cream (Product A), an alcohol-based gel cream (Product B), and a liquid toner (Product C). Eight microliters of either Product A or B was used. In the case of liquid Product C, 20 µl was used. These dosages resulted in a total deposition of SA at the surface of Product A (0.12 mg/cm^2) and Product B (0.11 mg/cm^2), and, for the liquids (Product C), 0.14 mg/cm^2.

Spectra were acquired using a RiverD gen2-SCA *in vivo* confocal Raman spectrometer, equipped with a 785-nm laser source. Laser power was 20 mW. The acquisition time for each spectrum was 5 s. The step size for depth profiles was 2 (Product C) or 3 µm (Products A and B). Each depth profile was recorded in triplicate at each time point and averaged for each volunteer (see below).

DATA ANALYSIS

Normalized intensities as illustrated in Figure 2A and B were calculated by dividing a peak area of an SA band by an internal intensity standard. SA exhibits an aromatic ring-breathing mode near 1040 cm^{-1}. This spectral feature was chosen because it is not shifted by the ionization state of SA and is sharp, intense, and well defined. Internal intensity standards are used to compensate for differences in intensity arising from experimental variables such as laser power, optical contact, and skin tone. One of these, used for Formulae A and B, is the ring-breathing mode of phenylalanine at 1000 cm^{-1}. The second, used to evaluate the liquid astringent Formula C, is the protein Amide I mode near 1650 cm^{-1}. The use of Amide I for Formula C was preferred due to a spectral overlap of ingredients with the phenylalanine band. To estimate the fraction of SA present in a given depth, the differences of the above ratios from the corresponding ratio at baseline were first calculated at each depth and time point. As depth profiles were recorded in triplicate, the baseline ratios were averaged before the differences from baseline were calculated. The differences from baseline for each time point and depth was averaged across volunteers. For a given depth profile, the differences from baseline were summed over the depths sampled. The sum represents all SA. The fraction of SA at a given depth was then the ratio of the difference from baseline at a given depth to the sum of differences from baseline.

Analysis of variance was used to establish statistical significance (Minitab™, Minitab, Inc., State College, PA).

RESULTS AND DISCUSSION

A representative *in vivo* Raman penetration profile is shown in Figure 1.

The responses of the band ratio of the SA band to that of Phe are shown in Figure 2A and B for a placebo and a SA-containing formula, respectively. The ratios are mapped out by the response surfaces, for which the x is depth, y time, and z the ratio of the SA band to

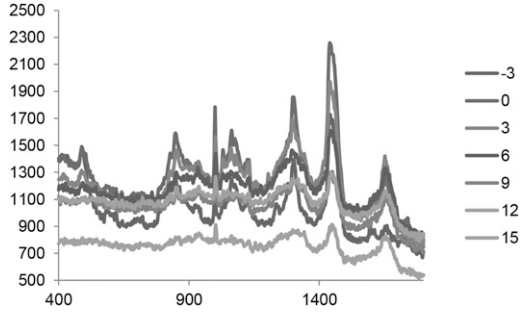

Figure 1.

that of the internal standard. The application of the product is indicated by the sharp rise in SA level at the first time point measured (0.5 h) at a depth of 0 μm (the surface). In contrast, the plot for the placebo is relatively flat.

The penetration results for each formula at individual time points are given in Figure 3A and B for the skin surface and at a depth of 6 μm, respectively. Data points are means. Error bars are expressed as standard deviation. There were no significant differences observed at the surface between formulae at the different time points. At a depth of 6 μm, it was observed that Formula B, a gel, exhibited significant differences from the liquid toner (Formula C) at 0.5 h after application and from Formulae A and C at 4 h after treatment.

Ethanol is known to enhance the penetration into skin. It accomplishes this function by several means. First, it may increase the solubility of the active, in this case SA, in the formula. SA is sparingly soluble in water, but is much more soluble in ethanol. Once in the stratum corneum, ethanol can interact with lipids and proteins. It can, e.g., hydrogen bond via the polar -OH group to the polar lipid head groups, disrupting the lateral packing of the lipids. In concert with the increased solubility of SA with ethanol in the nonpolar regions, penetration can be enhanced in the stratum corneum. This mechanism explains why the proportion of SA deposited is higher at the 6-μm depth in the case of Product B. The liquid phase containing SA in Products A and C–E is composed of water and NaOH, with 10% ethanol in Product C; in contrast, the corresponding phase in Product B contains 40% ethanol as the solvent. It can be proposed that on application of either Products A or C–E, between 63% and 80% SA precipitated at the skin surface, where it remained over the remainder of the time of interest in this work. This corresponds to

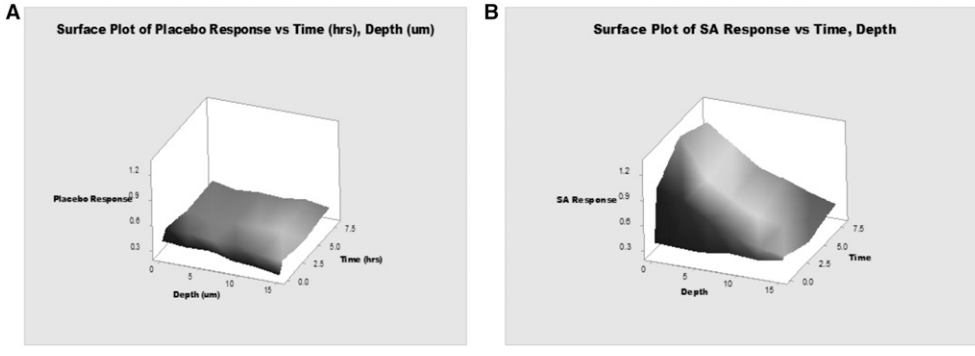

Figure 2.

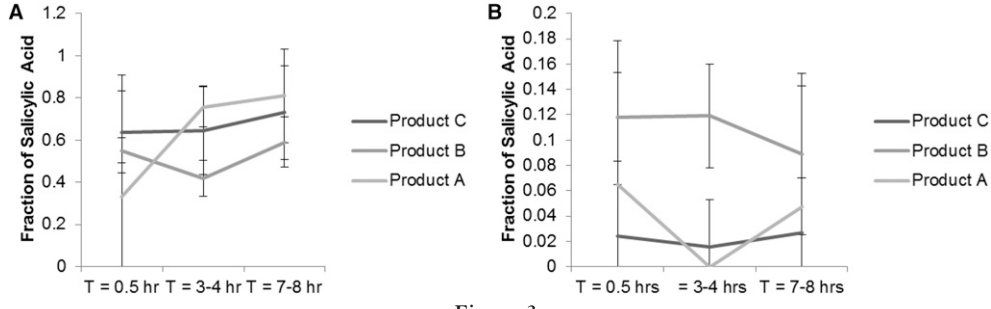

Figure 3.

0.08 and 0.11 mg/cm^2, respectively. The remainder was located at depths less than 6 μm. Slightly less of SA in Product B deposited at the surface, though the difference is not statistically significant. In contrast, a significantly higher fraction of SA, about 12% (0.013 mg/cm^2) penetrated to the 6-μm depth over the course of the first 4 h, the rest being distributed between the surface and 6 μm. The corresponding amounts for Products A and C–E were 0% and 2%, respectively. Deeper penetration after 4 h may not have occurred in the case of product B because the ethanol eventually evaporated or otherwise dissipated in the stratum corneum. Lowered ethanol content with time would result in a lowered solubility of SA and increasing lateral packing efficiency of the lipids, preventing further penetration. Lowered ethanol content with time would result in a lowered solubility of SA and increasing lateral packing efficiency of the lipids, preventing further penetration. Other specific interactions within the product matrix are expected to be important. For example, the relative amount of binding to polymers by SA and ethanol can influence the availability of either one of these.

CONCLUSION

Penetration differences of SA into skin for different antiacne formulae have been observed *in vivo* in human subjects using confocal Raman spectroscopy. The penetration distribution in the stratum corneum for an antiacne moisturizing cream and for low-alcohol (10%) toners were similar, with most of the SA remaining on the skin surface. Virtually no penetration was evident at a depth of 6 μm. In the case of a 40% alcohol-containing gel, however, 12% of the SA had penetrated to a depth of 6 μm after 4 h. These results are consistent with the known penetration-enhancing properties of ethanol. However, the matrix in which SA is contained, how SA binds to this matrix, and pH, are also important.

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