Impact of Order of Application of Moisturizers on Percutaneous Absorption Kinetics: Evaluation of Sequential Application of Moisturizer Lotions and Azelaic Acid Gel 15% Using a Human Skin Model

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The medical management of rosacea increasingly has involved not only the appropriate selection of topical medication but also patient education and specific recommendations regarding appropriate skin care. The recognition that epidermal barrier dysfunction and transepidermal water loss (TEWL) play a pathophysiologic role in rosacea and that skin moisturization may help to mitigate signs and symptoms of the disease has led to a deeper appreciation of the importance of proper skin care in the treatment of rosacea. Data from a percutaneous penetration study performed using human skin suggest that any of the tested moisturizer lotions may be applied either before or after azelaic acid gel 15% without a major change in the percutaneous absorption profile of azelaic acid.

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Rosacea is a common facial skin disorder estimated to affect at least 14 million people in the United States. Adults are predominantly affected, with most cases diagnosed before the age of 50 years.[1] The papulopustular subtype of rosacea generally is characterized by facial inflammatory lesions (papules, pustules), erythema, and telangiectases, and many patients are affected by symptoms such as burning and stinging. In most cases of rosacea, periods of spontaneous remission may alternate with periods of exacerbation.[1,2] Current medical treatment strategies include using topical therapies, such as sodium sulfacetamide–sulfur, metronidazole, and azelaic acid, or oral therapies, such as tetracycline derivatives, to control signs and symptoms of rosacea and mitigate flares.[1-3] It has become increasingly recognized that epidermal barrier dysfunction predominantly affecting central facial skin is a contributing factor related to signs and symptoms of rosacea.[4-7] As a result, optimal medical management of rosacea incorporates appropriate selection of topical medication, patient education, and use of an appropriate skin care regimen.[3-5] Physical modalities such as intense pulsed light and appropriately selected lasers may be used for treatment of persistent diffuse erythema, telangiectases, and phymatous changes.[3]

How does moisturizer use provide therapeutic impact when incorporated into the treatment regimen for rosacea?

Some reports have suggested that the use of moisturizers can have a beneficial effect on skin disorders such as rosacea, acne vulgaris, and atopic dermatitis by improving the signs and symptoms associated with epidermal barrier dysfunction and enhancing hydration of the stratum corneum, which leads to barrier repair.[4-9] Epidermal barrier dysfunction, characterized by increased transepidermal water loss (TEWL), is an integral feature of rosacea and may explain the sensitive facial skin characteristic of the disorder.[10] Well-formulated moisturizers, particularly those moisturizers combined in the appropriate application sequence with certain active topical formulations, may help to minimize the effects of epidermal barrier dysfunction and increased TEWL, thereby reducing signs and symptoms of cutaneous irritation and facilitating epidermal barrier repair.[5] Moisturizers replenish depleted lipids within intercellular lipid membranes of the epidermis and thus restore the ability of the stratum corneum to retain moisture.[4-9] A properly selected skin care regimen inclusive of a moisturizer used in combination with a topical medication also may reduce potential tolerability reactions that can be associated with topical therapy and may enhance percutaneous delivery of the active agent.[6-11] However, there are no prior data available evaluating how the order of moisturizer application affects percutaneous absorption of the active ingredient when the moisturizer is applied sequentially with topical medications for rosacea.

What measures can be used to assess the potential impact of moisturizer application on the percutaneous absorption kinetics of topical medications?

The in vitro human ex vivo skin model has proven to be a valuable tool for the study of percutaneous absorption and the determination of the pharmacokinetics of topically applied drugs.[11] The model uses human ex vivo skin mounted in specially designed diffusion chambers that allow the skin to be maintained at a temperature and humidity matching typical in vivo conditions.[10,11] A finite dose (eg, 4–7 mg/cm^2) of a formulation, which may include both the therapeutic agent and a moisturizer, is applied to the outer surface of the skin and drug absorption is measured by monitoring its rate of appearance in the reservoir solution that bathes the inner surface of the skin.[11] Data defining total absorption, rate of absorption, and skin content can be accurately determined in this model.[11,12] The method has a historic precedent for accurately predicting in vivo percutaneous absorption kinetics.[12,13]

How was the impact of order of application of a moisturizer on the percutaneous absorption kinetics of azelaic acid evaluated using the in vitro human ex vivo skin model?

An in vitro human skin penetration study was performed to determine the percutaneous absorption pharmacokinetics of azelaic acid gel 15% that incorporated radiolabeled azelaic acid (^{14}C-azelaic acid) applied before and after 3 different commonly recommended moisturizer lotions (Cetaphil® Moisturizing Lotion, Dove® Lotion, CeraVe™ Moisturizing Lotion). Azelaic acid gel 15% contains azelaic acid, a naturally occurring saturated dicarboxylic acid. Chemically, azelaic acid is 1,7-heptanedicarboxylic acid, with a molecular weight of 188.22. It is approved by the US Food and Drug Administration for the topical treatment of inflammatory papules and pustules of mild to moderate rosacea.[14]

The single-center, open-label, within-donor, human skin study evaluated azelaic acid gel 15% applied before and after the 3 different moisturizer lotions. The primary test formulation was azelaic acid gel 15%. Application of this formulation to the skin was conducted before and after the application of Cetaphil Moisturizing Lotion, Dove Lotion, or CeraVe Moisturizing Lotion. Each formulation was tested on skin from 3 different donors using the Franz finite dose model (see Study Procedures).

For the primary test formulation, matched sets of skin sections were either predosed with a lotion formulation before the application of azelaic acid or postdosed with a lotion formulation after the application of azelaic acid. All dose applications were 5 µL/cm^2 and were spread throughout the skin section. Fifteen minutes separated the first and second dosing applications to each skin section.

Calculated parameters included the amount absorbed (total penetration into the reservoir solution over 48 hours), rate of penetration (flux), and mass distribution (epidermal and dermal contents). Replicate skin sections within donors were averaged to provide a within-donor mean, which was used to calculate an across-donor population mean. Differences between the groups applying azelaic acid gel 15% before and after the lotion were evaluated using the Student *t* test. All conduct and data entry was reviewed and verified by quality control and quality assurance personnel as per the standard operating procedures of the testing facility.

What were the outcomes of the percutaneous absorption kinetics study evaluating moisturizer application with azelaic acid gel 15%?

The penetration profile observed for azelaic acid displayed a characteristic finite dose absorption profile with a rise to a peak rate of penetration followed by a slow but steady decline (Figure 1). The penetration profile for azelaic acid did not appreciably change

Study Procedures

Reagents and Source of Standards

All reagents used in this study were of analytical reagent grade or better.

Radiolabeled azelaic acid, [1-14C], was concentrated and mixed into the azelaic acid formulation to achieve a final specific activity of approximately 0.1 μCi per applied dose.

Diffusion Cell and Skin Preparation

Donated human trunk skin without obvious signs of skin disease was used in this study. At collection, skin samples were dermatomed, cryopreserved, sealed in a water-impermeable plastic bag, and stored at the appropriate temperature (approximately −70°C) until the day of the study.

Skin sections were mounted onto 1.0-cm^2 static Franz diffusion cells. The dermal reservoir solution was phosphate-buffered isotonic saline (pH, 7.4±0.1), and the epidermal chamber was left open to ambient laboratory environment. The cells were stirred magnetically at approximately 600 rpm and maintained to achieve a skin surface temperature of 32.0°C±1.0°C.

To assure the barrier integrity of each skin section, permeability to tritiated water was determined before application of the test products.[14] Skin specimens in which absorption of ^3H_2O from a 5-minute pulse exposure dose was less than 1.56 μL Eq/cm^2 were considered acceptable.

Dermal reservoir samples were collected at 2, 4, 6, 8, 12, 24, 32, and 48 hours after dose application. The reservoir solution was removed in its entirety and replaced with fresh reservoir solution, and a predetermined-volume aliquot was saved for subsequent analysis.

At 48 hours after dose application and after the last reservoir sample was collected, the skin surface was washed twice (0.5 mL each time) in an ethanol to water volume ratio of 50 to 50. Following the wash, the skin was removed from the chamber; the stratum corneum was collected by tape stripping, and the skin was split into epidermis and dermis and dissolved using a tissue solubilizer.

Analytic Laboratory

^{14}C-Azelaic Acid—Samples for ^{14}C were quantified for ^{14}C-azelaic acid content by liquid scintillation spectroscopy using a liquid scintillation counter. Each sample was counted for 5 minutes, in duplicate. Counts per minute were automatically converted to decays per minute using the external standard quench correction method. Decays per minute results were converted to mass amounts using the measured specific activity of the final dose formulation.

when dosed either before or after the 3 moisturizer lotions. Azelaic acid demonstrated extensive absorption through the skin with greater than 70% (±4%) of the applied dose being measured in the reservoir solution across all diffusion chambers.

For all the parameters measured (ie, total absorption, flux, epidermal and dermal contents), values were not found to be statistically significantly different (using the Student *t* test [$P>.05$]) when azelaic acid was applied before or after any of the 3 moisturizer lotions that were tested.

For 2 of 3 moisturizer lotions (Dove Lotion and CeraVe Moisturizing Lotion), the application sequence of lotion before azelaic acid gel 15% resulted in a trend toward greater percutaneous penetration of the active agent versus the application sequence of lotion after azelaic acid gel 15% (either Dove Lotion or CeraVe Moisturizing Lotion). This trend was most evident in the earlier penetration period (1–7 hours). The trend toward greater administered dose penetration with the lotion before active agent application sequence was not evident with Cetaphil Moisturizing Lotion.

The application sequence of Dove Lotion before azelaic acid gel 15% showed an average total penetration of applied dose (for all 3 skin donors) of 424.5 (±20.6) μg/cm^2 versus 402.4 (±29.1) μg/cm^2 for the application sequence of Dove Lotion after azelaic acid gel 15%. Total penetration of applied dose by percentage for the Dove Lotion before azelaic acid application sequence was 70.7% versus 67.0% for the Dove Lotion after azelaic acid application sequence.

The greatest between-sequence difference in penetration rates was seen early in the testing duration at 1, 3, 5, and 7 hours (midtime of sample collection periods)(Figure 2). At 1 hour, the Dove Lotion before azelaic acid application sequence showed an average penetration rate of 22 μg/cm^2/h versus 12 μg/cm^2/h for the Dove Lotion after azelaic acid application sequence. These differences increased at 3 hours to 49 μg/cm^2/h versus 34 μg/cm^2/h, respectively. By 7 hours, the difference began to minimize and was 28 μg/cm^2/h for the

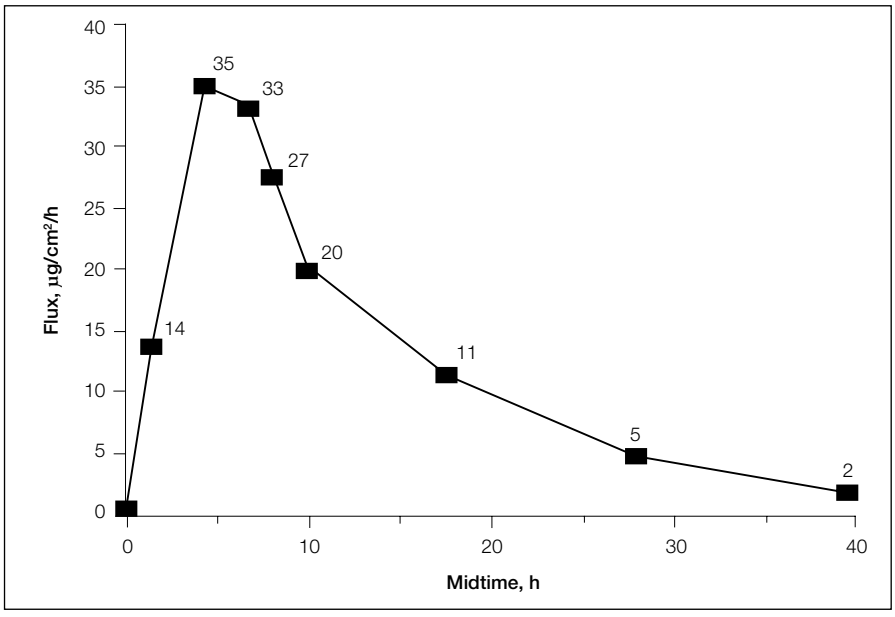

Figure 1. Overall rate of penetration profile for azelaic acid gel 15% versus midtime of sample collection period.

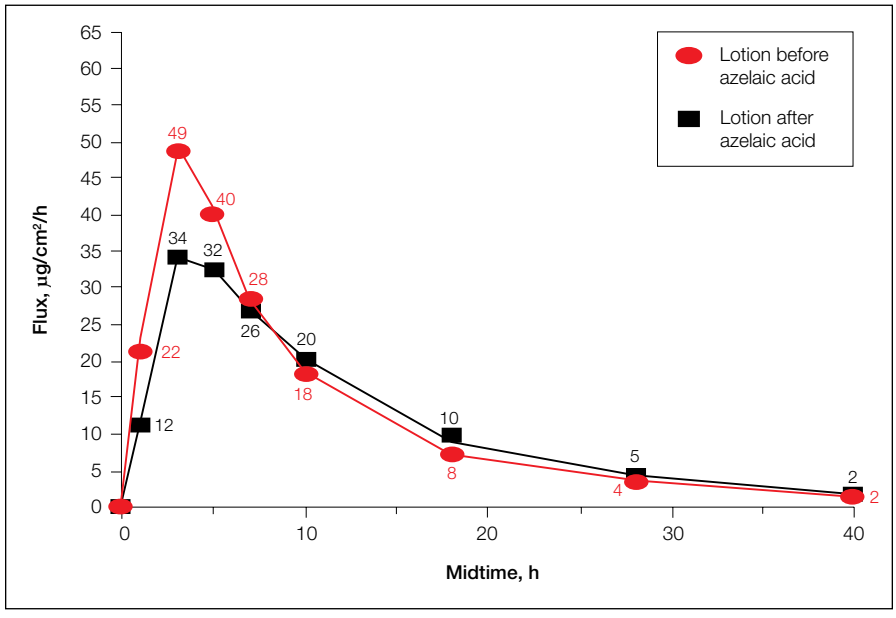

Figure 2. Penetration profile for azelaic acid gel 15% when used with Dove® Lotion.

Dove Lotion before azelaic acid application sequence versus 26 µg/cm²/h for the Dove Lotion after azelaic acid application sequence. Epidermal and dermal concentrations and percentages of azelaic acid penetration were comparable across both application sequences.

For the application sequence of CeraVe Moisturizing Lotion before azelaic acid gel 15%, the average total penetration of applied dose (for all 3 skin donors) was 431.9 (±20.3) µg/cm² versus 381.7 (±47.0) µg/cm² for the application sequence of CeraVe Moisturizing Lotion after azelaic acid gel 15%. Total penetration of applied dose by percentage for the CeraVe Moisturizing Lotion before azelaic acid application sequence was 71.9% versus 63.5% for the CeraVe Moisturizing Lotion after azelaic acid application sequence.

The greatest between-sequence difference in penetration rates was seen early in the testing duration at the 1, 3, 5, and 7 hours midtime sample collection periods (Figure 3). At 1 hour, the CeraVe Moisturizing Lotion before azelaic acid application sequence showed an average penetration rate of 9 µg/cm²/h versus 18 µg/cm²/h for the CeraVe Moisturizing Lotion after azelaic acid application sequence. These differences increased at 3 hours but favored the CeraVe Moisturizing Lotion before azelaic acid application sequence (39 µg/cm²/h vs 29 µg/cm²/h). By 7 hours, the difference favoring the CeraVe Moisturizing Lotion before azelaic acid application sequence was 32 µg/cm²/h versus 22 µg/cm²/h for the CeraVe Moisturizing Lotion after azelaic acid application sequence. Epidermal and dermal

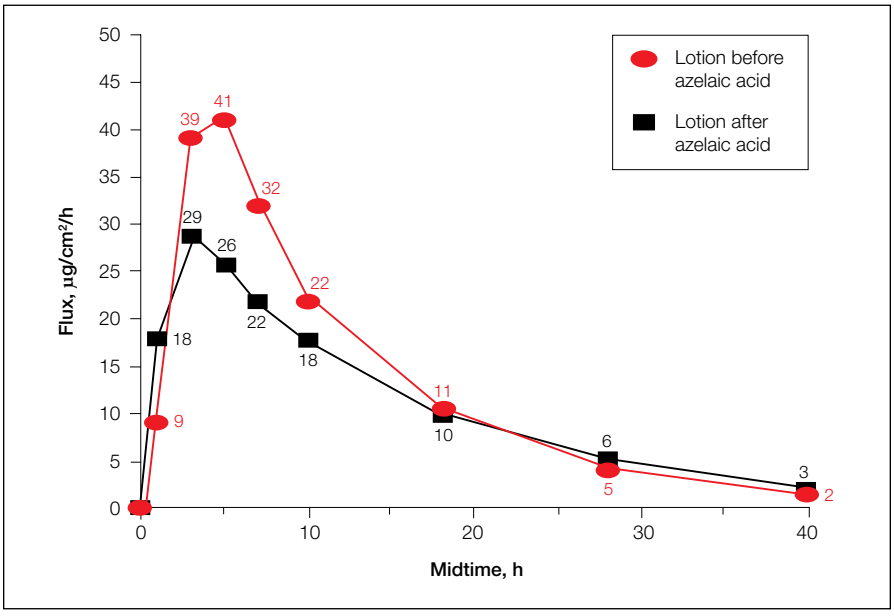

Figure 3. Penetration profile for azelaic acid gel 15% when used with CeraVe™ Moisturizing Lotion.

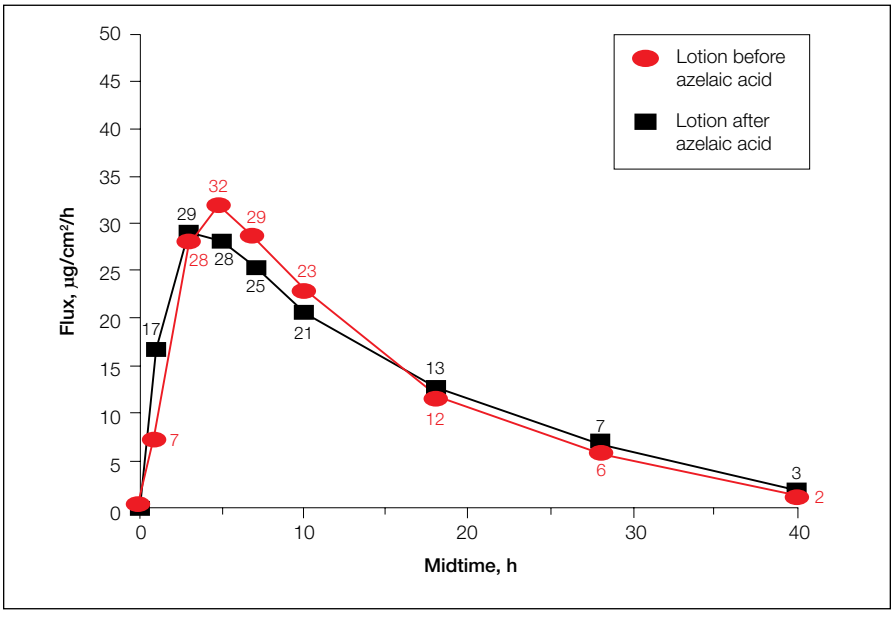

Figure 4. Penetration profile for azelaic acid gel 15% when used with Cetaphil® Moisturizing Lotion.

concentrations and percentages of azelaic acid penetration were comparable across both application sequences.

The combination of azelaic acid and Cetaphil Moisturizing Lotion demonstrated little difference in application sequence penetration profiles of azelaic acid (Figure 4).

What is the clinical significance of the results obtained from the percutaneous absorption kinetics study completed with application of specific moisturizer lotions either before or after azelaic acid gel 15%?

Because moisturizer use is commonly recommended as part of the management of skin disorders such as rosacea and acne vulgaris, it is important to investigate the sequential application of topical medications and moisturizers regarding impact on percutaneous absorption of the active ingredient. When there are sequential applications of 2 or more topical formulations, the risk of intermixing the different vehicle matrices may result in unpredictable percutaneous delivery of active ingredients. This study individually evaluated the impact of order of application of 3 moisturizer lotions (Cetaphil Moisturizing Lotion, Dove Lotion, CeraVe Moisturizing Lotion) on the percutaneous absorption of azelaic acid formulated in its aqueous gel vehicle (azelaic acid gel 15%).

The data from this study indicate that the sequence of application of azelaic acid gel 15% and any of the

3 moisturizer lotions did not markedly alter the pharmacokinetic delivery of azelaic acid into human skin, which is noteworthy from a clinical perspective in that the potential for markedly diminished percutaneous delivery of azelaic acid was not evident regardless of whether the moisturizer lotion was applied before or after azelaic acid gel 15%. The data did suggest that azelaic acid percutaneous absorption may be increased by some moisturizers that are applied before azelaic acid gel 15%.

The percutaneous penetration of azelaic acid did not appear to be compromised by application either before or after any of the 3 moisturizer lotions that were tested. However, this observation may not be automatically applicable to other moisturizer formulations that are sequentially applied with azelaic acid or any other topical medications that have not been tested. Additionally, it may be that increasing the duration between the application of the active agent and the lotion, or vice versa, could further diminish the chance of unintended alteration of the percutaneous absorption kinetics of the active ingredient.

Conclusion

The medical management of rosacea increasingly has involved not only the appropriate selection of topical medication but also patient education and specific recommendations regarding appropriate skin care. The recognition that epidermal barrier dysfunction and TEWL play a pathophysiologic role in rosacea, and that skin moisturization may help to mitigate signs and symptoms of the disease, has led to a deeper appreciation of the importance of proper skin care in the treatment of rosacea.

In this in vitro Franz diffusion cell human skin evaluation of the penetration profile of azelaic acid gel 15% applied either before or after 1 of 3 moisturizer lotions, the between-sequence difference did not reach statistical significance. For 2 of 3 moisturizer lotions (Dove Lotion and CeraVe Moisturizing Lotion), there was a trend toward greater percutaneous penetration and mass distribution of the active ingredient (azelaic acid) observed with the application sequence of the moisturizer lotion applied before the azelaic acid gel 15%.

The data presented in this study suggest that any of the tested moisturizer lotions may be applied either before or after azelaic acid gel 15% without a marked alteration in percutaneous penetration of azelaic acid. Although effect on efficacy was not evaluated in this study, it is anticipated that there would not be reduction in efficacy of azelaic acid gel 15% with sequential application of any of the tested moisturizer lotions regardless of order of application, as there was no major alteration in delivery of azelaic acid into the skin and the time course of skin penetration of azelaic acid was not substantially altered. Because moisturizer use is recommended in the treatment of rosacea, the information gleaned from this study is likely to be clinically applicable.

REFERENCES

1. Del Rosso JQ. Medical treatment of rosacea with emphasis on topical therapies. *Expert Opin Pharmacother*. 2004;5:5-13.
2. Bikowski JB. The pharmacologic therapy of rosacea: a paradigm shift in progress. *Cutis*. 2005;75(suppl 3):27-32.
3. Bikowski JB, Goldman MP. Rosacea: where are we now? *J Drugs Dermatol*. 2004;3:251-261.
4. Del Rosso JQ. Adjunctive skin care in the management of rosacea: cleansers, moisturizers, and photoprotectants. *Cutis*. 2005;75(suppl 3):17-21.
5. Del Rosso JQ. The role of skin care and maintaining proper barrier function in the management of rosacea. *Cosmet Dermatol*. 2007;20:485-490.
6. Draelos ZD. Concepts in skin care maintenance. *Cutis*. 2005;76(suppl 6):19-25.
7. Bikowski J. The use of therapeutic moisturizers in various dermatologic disorders. *Cutis*. 2001;68(suppl 5):3-11.
8. Lebwohl M, Herrmann LG. Impaired skin barrier function in dermatologic disease and repair with moisturization. *Cutis*. 2005;76(suppl 6):7-12.
9. Ghali FE. Improved clinical outcomes with moisturization in dermatologic disease. *Cutis*. 2005;76(suppl 6):13-18.
10. Dirschka T, Tronnier H, Folster-Holst R. Epithelial barrier function and atopic diathesis in rosacea and perioral dermatitis. *Br J Dermatol*. 2004;150:1136-1141.
11. Franz TJ. Percutaneous absorption on the relevance of in vitro data. *J Invest Dermatol*. 1975;64:190-195.
12. Franz TJ. The finite dose technique as a valid in vitro model for the study of percutaneous absorption in man. In: Simon GA, Paster Z, Klingberg MA, et al, eds. *Skin: Drug Application and Evaluation of Environmental Hazards, Current Problems in Dermatology*. Vol 7. Basel, Switzerland: S Karger; 1978:58-68.
13. Franz TJ, Lehman PA. The use of water permeability as a means of validation for skin integrity in in vitro percutaneous absorption studies [abstract]. *J Invest Dermatol*. 1990;94:525.
14. Finacea [package insert]. Pine Brook, NJ: Intendis, Inc; 2005.