Development and Clinical Analysis of a Novel Humectant System of Glycerol, Hydroxyethylurea, and Glycerol Quat

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The clinical benefits of glycerol as a skin humectant are well understood; however, glycerol has intrinsic limitations. Investigation into novel moisturizers presents an opportunity to both supplement glycerol and provide hydration to layers of stratum corneum (SC) not currently addressed by available agents.

The objectives of this study were (1) to utilize quantitative modeling of humectant physicochemical properties to identify moisturizing molecules to complement glycerol and hydrate all layers of the SC; and (2) to assess the clinical performance of a novel humectant combination containing glycerol, hydroxyethylurea, and glycerol quat (a novel humectant) in increasing skin hydration.

Methods to assess hydration efficacy of the proprietary humectant mixture included visual grading, skin conductance and capacitance, transepidermal water loss, and confocal Raman spectroscopy.

Short-term and long-term studies revealed significantly greater improvements in skin hydration throughout the SC with the combination of humectants compared with glycerol alone. Additionally, the humectant mixture significantly reduced eczema-associated dryness with 4 weeks' of use.

Two humectants, hydroxyethylurea (HEU) and glycerol quat (GQ), were identified to complement the hydration achieved by glycerol and to moisturize all layers of the SC. Both short-term and long-term use of the combination humectant system resulted in greater improvements in multiple quantitative measures of skin hydration. The novel humectant combination led to sustained improvements in clinical dryness over any 1 ingredient alone, indicating a synergism among the 3 humectants in hydrating the SC. The clinical performance of this humectant mixture in unaffected, extremely dry, and diseased skin represents a promising new development in topical moisturizers.

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Maintaining proper water content in the stratum corneum (SC)—the top layers of the epidermis—is known to be critical not only for skin plasticity and visual appearance, but also for proper desquamation, formation of natural moisturizing factors, and other enzymatic processes in the epidermis.[1-4] Moisturizers are commonly used to protect skin from dryness and as dermatologic and cosmetic skin therapies. Skin moisturizers typically contain various combinations of humectants, occlusives, and emollients to achieve beneficial effects.[5-7] Humectants attract water to the SC, increasing its water content, and occlusives provide a barrier to water transport from skin to the environment, reducing transepidermal water loss (TEWL) and increasing water activity at the skin surface. The trihydroxylated glycerol molecule is considered to be the most effective humectant for dry skin.[3,8-10] Petrolatum, the most widely used occlusive agent, was introduced to skin care in 1872. Humectants and occlusives often work more effectively to increase SC hydration when properly combined; thus, the combination of glycerol and petrolatum has been the mainstay of skin moisturizers for over half of the last century. This synergic effect is more pronounced in low-humidity environments (N. Lu, PhD; unpublished data; December 2006).[5-7,11]

Nevertheless, although highly effective, the glycerol/petrolatum combination is not the ideal skin moisturizer. For example, alleviation of severe dry skin or dry skin under very low humidity conditions requires products containing high concentrations of petrolatum, glycerol, or both. However, such formulations are aesthetically unpleasant because they are highly viscous and oily. Over the years, novel moisturizers with improved efficacy for dry skin and better in-use sensory properties have actively been sought. Progress, however, has been limited.

We have performed theoretical analyses and mathematical modeling of skin moisturization to deepen our understanding of moisturization mechanisms and explore opportunities for developing better skin-moisturizer technologies (N. Lu, PhD; unpublished data; December 2006). We have analyzed the humectant efficacy of glycerol, its intrinsic limitations in hydrating skin, and potential strategies to overcome such limitations. Consequently, we have developed a novel humectant system, a mixture of carefully selected molecules, including a proprietary molecule (glycerol quat [GQ]), that outperforms current glycerol-based moisturization systems. Short-term (4-hour) skin-moisturization clinical studies were conducted to compare the performance of the new humectant system with that of glycerol. Longer term clinical studies also were performed to study the effectiveness of the humectant system in a fully formulated moisturizing lotion for the treatment of dry skin caused by eczema and winter xerosis.

MOISTURIZATION MECHANISMS AND INSIGHTS FROM MODELING

We have developed a mechanistic model based on principles of thermodynamics, transportation and kinetics theories, and the structure and properties of the SC and viable skin layers (N. Lu, PhD; unpublished data; December 2006). The in-use moisturization effect of a humectant is determined by the physicochemical properties of the molecule, composition and properties of the product formulation, SC properties, environmental conditions, and moisturizer application. To be effective and long lasting, a humectant should permeate and remain within the corneocytes long enough to increase local hydration before diffusing further into the skin. This requires a proper balance of several key physicochemical properties of the molecule, including molecular weight, lipophilicity (water-octanol partition coefficient [$K_{o/w}$]; the ability of a molecule to penetrate the SC), water sorption isotherm (water absorption capacity over a range of relative humidities at constant temperature), and solubility. This concept differs from the traditional view that high hygroscopicity is the main attribute required for an effective humectant.

Glycerol, although only moderately hygroscopic, possesses well-balanced physicochemical properties that make it an effective skin humectant with nearly unmatched clinical performance. Molecules such as magnesium chloride, although highly hygroscopic, are not effective skin humectants, likely due to a low $K_{o/w}$ (Table). Small molecules, such as butylene glycol, have favorable partition coefficients for permeating the SC, but are less likely to partition into the corneocytes, thus limiting in vivo moisturization.

Sagiv et al[12] compared monohydroxylated, dihydroxylated, and trihydroxylated glycerol application in reducing clinical dryness and erythema and concluded that glycerol was the most effective, although not the most hygroscopic, humectant. This finding is consistent with our understanding of the relationship between in vivo humectant performance and molecule properties and clinical studies of select humectant systems (Figure 1).

Another important insight suggested by modeling is that, for a given humectant molecule, the profile of hydration effect across the SC depth is determined by the physicochemical properties mentioned above. Thus, a single type of humectant cannot hydrate different depths of the SC equally because of the nature of diffusion and water absorption. In other words, the moisturization effect of a single type of humectant is not uniform across

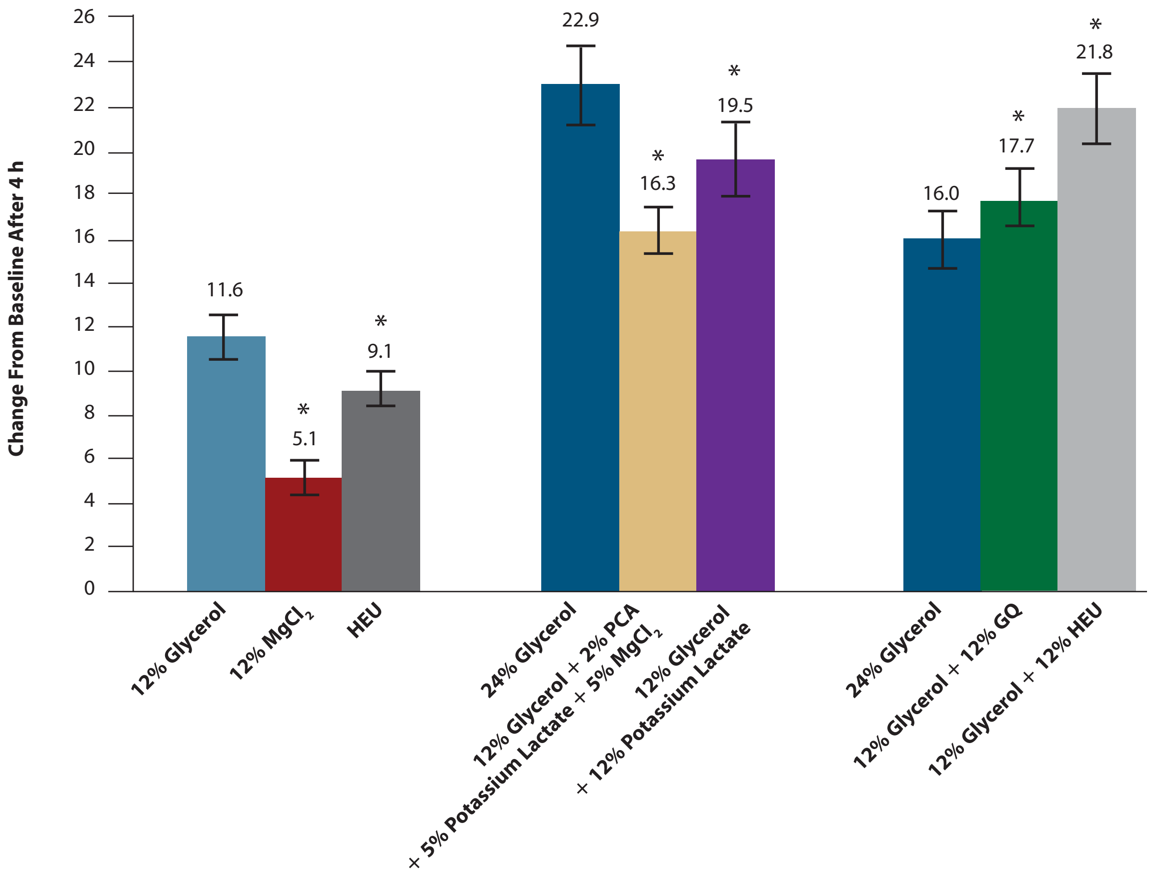

Figure 1. Clinical performance of aqueous solutions of glycerol versus selected humectants and humectant combinations. Change from baseline in skin capacitance measurements (Corneometer CM 825 skin hydration meter) reported 4 hours following single application of humectant(s). Asterisk indicates $P \leq .05$ compared with glycerol. HEU, indicates hydroxyethylurea; PCA, pyrrolidone carboxylic acid; GQ, glycerol quat.

the depth of the SC. In topical moisturizers, glycerol hydrates the middle and deep layers of the SC well; however, the hydration efficacy is lower in the surface layers of the SC, where moisturization is most needed (N. Lu, PhD; unpublished data; December 2006). This is an intrinsic limitation of glycerol. Traditional glycerol/occlusive combinations partially overcome the hydration deficit at the surface layers of the SC, as can increasing the glycerol content (Figure 1); however, high concentrations of glycerol are associated with negative aesthetics, such as stickiness. These limitations suggested an opportunity to design a humectant system to more effectively hydrate all the layers of the SC.

DEVELOPMENT OF A NOVEL HUMECTANT SYSTEM

Although a single humectant that can adequately hydrate throughout the SC has not yet been identified, it may be possible to achieve hydration of all layers using a combination of humectants with differing physicochemical properties. A proprietary mixture of glycerol, hydroxyethylurea (HEU), and the novel humectant GQ (Figure 2), designed to supplement and potentially enhance the hydration efficacy of glycerol, is described here. Hydroxyethylurea was selected for inclusion because of its miscibility with glycerol and increased lipophilicity compared with glycerol ($K_{o/w}$ −1.2 vs −1.7)(Table), suggestive of more rapid penetration of the SC.

The third component of the mixture is GQ, a quaternized derivative of glycerol that is completely miscible with glycerol and more hydrophilic ($K_{o/w}$ −2.8 vs −1.7) (Table). As a consequence of its approximately 60-fold lower partition coefficient than glycerol, when delivered in combination, GQ diffuses into SC at a slower rate than glycerol or HEU and thus is better able to hydrate the surface layers of the SC. Glycerol quat also possesses greater water sorption ability than glycerol (N. Lu, PhD; unpublished data; December 2006), allowing for superior hydration at the drier surface layers. Thus, it may be possible to hydrate different layers of the SC in a balanced manner with a combination of humectants, in this case glycerol, HEU, and GQ. This novel concept of thoroughly hydrating different levels of the SC using humectants with different physicochemical properties implies a dynamic distribution, or stratification, of moisturizing ingredients within the SC. We have conducted a series of clinical studies to investigate the effectiveness of this novel humectant mixture in a fully formulated lotion on xerotic and eczematic skin.

MATERIALS AND METHODS

Clinical studies were conducted to (1) assess the moisturization performance of the 3-component humectant mixture and (2) evaluate the effectiveness of a lotion containing the humectant mixture for treating winter xerosis and eczema-associated dry skin. All studies were approved by an independent institutional review board.

Sample Preparation

Aqueous solutions of glycerol, HEU, GQ, or the proprietary humectant mixture were prepared at 20% (wt%) in deionized water. Fully formulated lotions were prepared with either 20% glycerol or 20% proprietary humectant system, both in a conventional oil-in-water base consisting of emulsifiers, structuring polymers, sensory particles, silicones, emollients, perfume, and preservatives.

Evaluation Methods

Visual dryness was assessed using the Kligman dry skin grading scale, where 0=none and 4=severe. Water content within the SC was determined by confocal Raman spectroscopy model 3510 (Skin Composition Analyzer). Skin hydration was determined using both conductance (Skicon-200EX skin hygrometer) and capacitance (Corneometer CM 825 skin moisture meter). Changes in barrier function were assessed by TEWL (DermaLab TEWL meter). Eczematous lesions were assessed using the Eczema Area and Severity Index at test sites, where 0=none, 1=mild, 2=moderate, and 3=severe.

Four-Day Study of Visual Dryness Reduction With Various Aqueous Humectant Solutions

Eighteen participants with visual dryness scores between 2 and 3.5 on their lower lateral legs were enrolled in this study. Two test sites were marked on each leg, and treatments were assigned in a randomized complete block design. Aqueous humectant solutions were applied twice daily to the test sites at an application rate of 2 mg/cm^2 and uniformly rubbed in by trained clinicians. The study was conducted in the prevailing dry skin conditions in Connecticut in March; the average external temperature during the study was 3°C. Dryness was evaluated each morning following a 30-minute equilibration period and immediately prior to the morning treatment application.

Confocal Raman Spectroscopy Study of Water Profiles Across the SC

Confocal Raman spectroscopy was conducted following a single application of a 20% aqueous solution of the humectant mixture or glycerol. Spectra using a 671 nm laser were obtained at baseline, 30 minutes postapplication, and 4 hours postapplication for each of 15 enrolled participants. Data from 3 marked forearm sites per participant were collected and averaged. Results were reported as percentage difference in water content between the humectant mixture and glycerol applications in top (0–4 µm depth), middle (4–12 µm depth), and deep (12–20 µm depth) SC regions.

Short-term (4-Hour) Moisturization Study of Fully Formulated Moisturizers

Fourteen participants with dry skin enrolled in this 4-hour, short-term skin-moisturization study. Upon enrollment, the forearms of all participants were

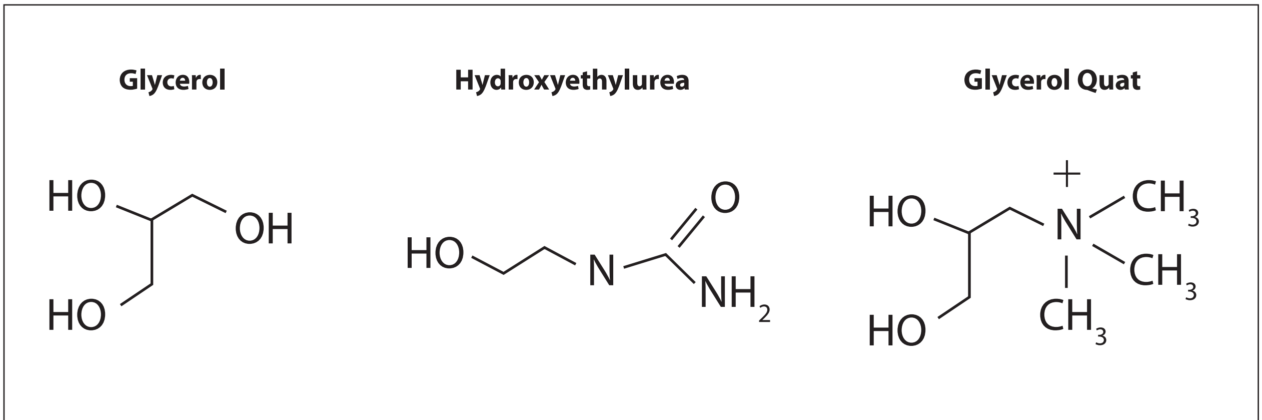

Figure 2. Molecular structures of glycerol, hydroxyethylurea, and glycerol quat.

Molecular Weight and Physicochemical Properties of Humectants Used in Skin Moisturizers[a]

Humectant	Molecular Weight	Lipophilicity	Hygroscopicity
Butylene glycol	90	−0.086	0.25
Propylene glycol	76	−0.59	0.32
Hydroxyethylurea	104	−1.2	0.25
Glycerol	90	−1.7	0.27
Glycerol quat chloride	169	−2.8	0.40
Sorbitol	182	−3.0	0.14
Potassium lactate	129	<−3	0.56
Sodium PCA	137	<−3	0.65
$MgCl_2·6H_2O$	204	<−3	0.58

Abbreviations: PCA, pyrrolidone carboxylic acid; $MgCl_2•6H_2O$, magnesium chloride hexahydrate.
[a]Lipophilicity calculated by the octanol-water partition coefficient ($K_{o/w}$) log. Hygroscopicity calculated as grams of H_2O bound by 1 g of humectant at 50% relative humidity.

"normalized" through controlled washing with bar soap (30 seconds of lathering followed by a 15-second rinse with tap water). Participants were acclimatized in ambient room conditions for 30 minutes, baseline hydration measurements were taken, then 2 mg/cm^2 of moisturizer product was applied to marked test sites on the forearm. Each participant received applications of all test products. Skin conductance and capacitance were evaluated at each test site at 1, 2, and 4 hours postapplication.

Treatment of Winter Xerosis With the Fully Formulated Moisturizer Containing Humectant Mixture

Changes in visual dryness in participants with winter xerosis were determined following treatment with a fully formulated lotion containing the proprietary mixture of glycerol, HEU, and GQ. Ninety-two participants with dry skin were enrolled in the 2-week treatment study (mean daily temperatures ranged from −7°C to 12°C), followed by a 3-day regression period during the early spring in Winnipeg, Canada. All participants first completed a 1-week conditioning phase during which they cleansed normally at home with a mild synthetic detergent cleansing bar. Following conditioning, participants were screened for baseline visual leg dryness. Participants with a dryness score between 1.5 and 2.5 were eligible for inclusion in the product-application phase of the study. All participants received a plain white pump bottle containing the test lotion and applied 1 pump (≈1.5 g) of test product to the leg twice daily for 2 weeks. During the product-application phase, participants continued to cleanse their test sites normally with the synthetic detergent bar. Use of other skin moisturizers was not permitted at any time during the study.

Four-Week Study of Humectant Lotion in Participants With Eczema

Ten participants with mild to moderate eczema on the arms or legs were enrolled in an evaluator-blinded, monadic, 4-week study. Participants received a plain white pump bottle of fully formulated lotion containing 20% proprietary humectant mixture and applied the test lotion at least twice daily for 4 weeks. Participants continued their existing eczema treatment regimens during the study. Dermatologist-assessed clinical evaluations of visual dryness were recorded at baseline, week 2, and week 4. Digital photographs of 1 affected and 1 unaffected site were taken at baseline, week 2, and week 4.

Statistical Analysis

For data analysis, paired *t* tests were used for all studies to assess the significance of differences in clinical performance. Statistical significance was set as $P \leq .05$ for all analyses.

RESULTS

Hydration Efficacy of an Aqueous Solution of Proprietary Humectant Mixture

The efficacy of a 20% aqueous solution of the humectant mixture was assessed by visual dryness over 4 days of twice-daily use (Figure 3). The proprietary humectant mixture resulted in significantly greater improvements from baseline in overall visual dryness than any individual component. Visual improvements with use of the mixture compared with 20% glycerol were significant at both day 3 (improvement from baseline, 1.58 vs 1.25) and day 4 (1.67 vs 1.33). Similarly, the humectant mixture was significantly more effective in reducing visual dryness than 20% GQ ($P < .0001$) or 20% HEU ($P < .0001$). The reductions in visual dryness due to application of 20% glycerol, GQ, or HEU were all similar at each time point and at the conclusion of the study (glycerol vs GQ, $P = .826$; glycerol vs HEU, $P = .579$; GQ vs HEU, $P = .521$).

Confocal Raman Spectroscopy Analysis of SC Water Content

Confocal Raman spectroscopy has emerged as an important tool for determination of water content across the depth of the epidermis and noninvasive assessment of skin-moisturizer efficacy. A representative confocal Raman spectroscopy hydration profile of the SC is shown in Figure 4A, indicating approximately 20% water per unit keratin at the surface of the SC and over 50% at a depth of 20 µm. Four hours posttreatment, both moisturizers increased the water content throughout the SC, including the surface layers (0–4 µm depth)(Figure 4B). Treatment with the humectant mixture resulted in a significantly greater increase in overall hydration of the SC than did 20% glycerol ($P < .05$). The greater increase in water content resulting from the mixture use was observed both at the skin surface and at the deeper SC layers.

Short-term (4-Hour) Moisturization Study of Fully Formulated Moisturizers

The fully formulated lotion containing 20% humectant mixture delivered significantly greater hydration to the skin following a single application than the lotion containing only 20% glycerol (Figure 5). The effect on skin conductance was significant as early as 1 hour postapplication and remained significant for the 4-hour postapplication period ($P < .0001$)(Figure 5A). Similar results were observed with capacitance analysis with use of the 20% humectant mixture lotion, resulting in significant improvements in hydration over 20% glycerol lotion ($P = .007$) beginning 1 hour postapplication (Figure 5B).

Treatment of Winter Xerosis With the Fully Formulated Moisturizer Containing Humectant Mixture

A 2-week product-application study was conducted to assess the performance of a fully formulated moisturizer containing 20% proprietary humectant mixture in

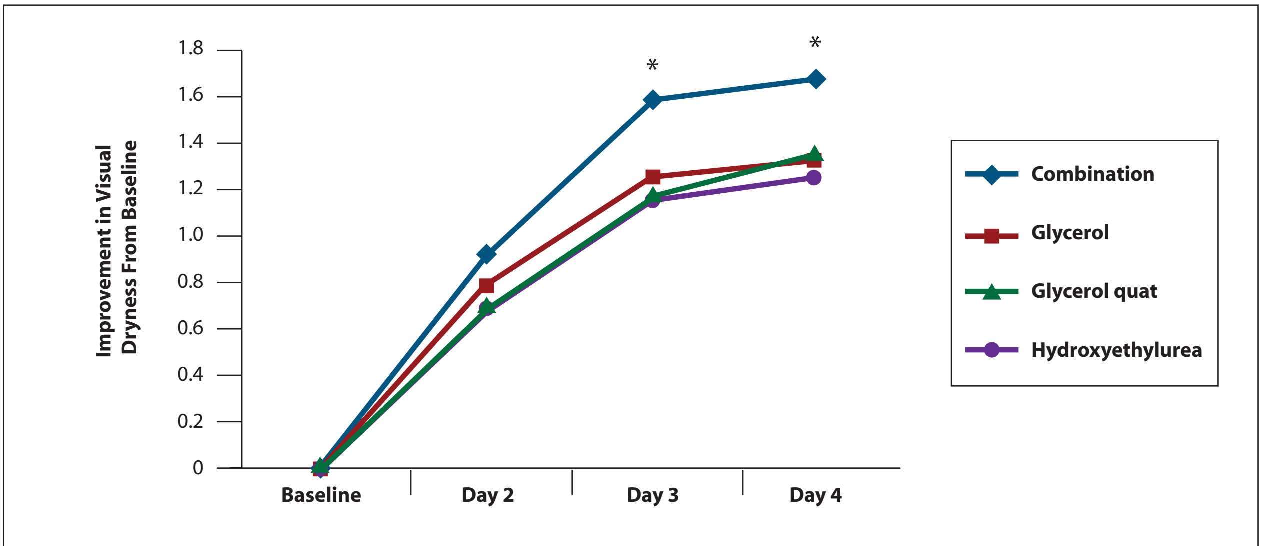

Figure 3. Clinical evaluation of visual dryness following application of aqueous humectant solutions. Comparison of clinical efficacy of 20% aqueous solutions of the humectant mixture, glycerol, glycerol quat, and hydroxyethylurea. Visual dryness was assessed using the Kligman dry skin grading scale, where 0=none and 4=severe. Data reported as improvement from baseline in dryness over 4 days. Asterisk indicates $P \leq .05$ compared with glycerol, glycerol quat, or hydroxyethylurea alone.

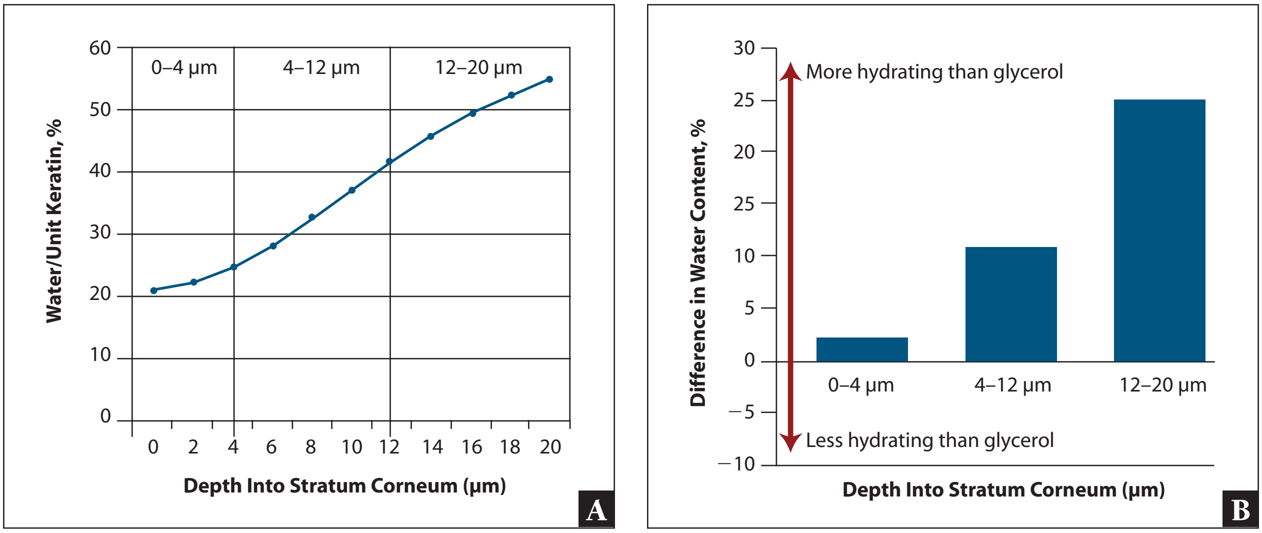

Figure 4. Confocal Raman spectroscopic analysis of stratum corneum hydration. Hydration calculated as percent water per unit keratin. Representative confocal Raman hydration profile of untreated stratum corneum (A). Improvement in stratum corneum hydration achieved at various depths with application of the 20% humectant mixture compared with 20% glycerol (B). Differential hydration shown as the absolute difference in improvement in water content between the humectant mixture and glycerol.

reducing clinical signs of winter xerosis and improving SC barrier function. At baseline, participants had moderate to severe leg dryness with a mean score (SD) of 2.47 (0.50). Treatment with the proprietary lotion resulted in a significant reduction in visual dryness at the first post-application measurement point (day 3) compared with baseline ($P<.001$)(Figure 6A). This reduction was sustained for the duration of the 2-week lotion application phase ($P<.001$ for all time points) despite the daily cleansing of the test sites and prevalent winter weather. All participants experienced reductions in visual leg dryness to a mean score (SD) of 1.03 (0.75)(slight dryness) by day 14. Consistent with the observed reduction in visual dryness, TEWL also was reduced following application of the lotion containing the humectant mixture (Figure 6B). The improvements over baseline were significant at days 3, 7, and 14 ($P<.001$ for all time points). Visual dryness and TEWL increased slightly during a 3-day regression period but remained significantly below baseline ($P<.001$ and $P<.05$, respectively).

Four-Week Study of Humectant Lotion in Participants With Eczema

Moisturizers are frequently used as adjunctive treatments to eczema therapy.[13] The moisturizing lotion containing 20% proprietary humectant mixture was evaluated as an adjunctive treatment for dry skin associated with mild to moderate

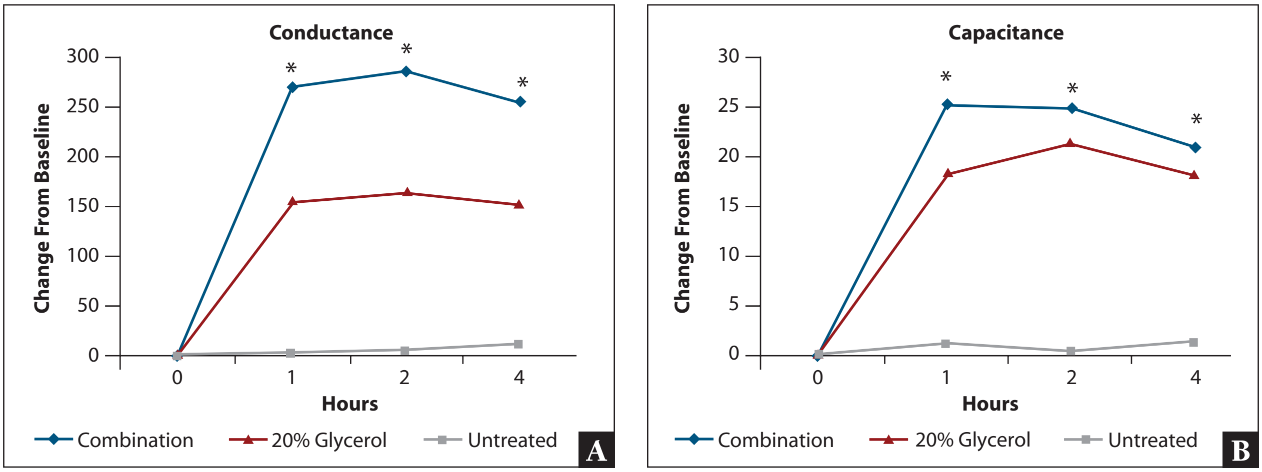

Figure 5. Four-hour hydration studies of fully formulated moisturizers. Comparison of clinical efficacy of formulated lotions containing 20% humectant mixture or 20% glycerol. Hydration assessed by conductance (A) and capacitance (B) and reported as change from baseline. Asterisk indicates $P\leq.05$ compared with glycerol.

eczema. After 4 weeks of treatment, there were significant reductions in Eczema Area and Severity Index scores compared with baseline (0.38 vs 0.72; P=.042)(Figure 7). The humectant mixture also resulted in visibly improved eczematous skin after 2 and 4 weeks of application.

DISCUSSION

Glycerol is a natural endogenous humectant in skin and has been well established as an effective topical moisturizer ingredient.[14,15] Previous work has established the inability of hygroscopicity to fully predict in vivo moisturization potential of a given humectant because the efficacy of glycerol as a topical moisturizer exceeds predictions based solely on its water-binding capacity.[12] Through theoretical and modeling studies,[9] we have concluded that molecular weight and lipophilicity of glycerol are 2 additional key determinants of in vivo hydration efficacy.

In general, formulated moisturizing systems relying on a single humectant would not be expected to hydrate the entire depth of the SC effectively because penetration into the SC is largely dependent on the physicochemical properties of the molecule.[16,17] Clinical analysis of a novel combination of glycerol, HEU, and GQ has provided direct in vivo support for the rationale of combining glycerol with selected humectants that have the physicochemical properties required to significantly improve skin hydration across all layers of the SC.

The humectant system reported here outperforms glycerol in in-use moisturization efficacy, as demonstrated in short-term moisturization clinical study and water profile measurement via confocal Raman spectroscopy. In addition to markedly improving visual dryness assessments and increasing water content within the skin, the humectant mixture significantly improved skin barrier function with longer term use in participants with dry skin. Furthermore, this new moisturizer is an effective adjunctive treatment for patients with mild or moderate eczema, indicating additional benefits in patients with highly compromised skin. The clinical benefits in treating both dry and diseased skin make this novel humectant system/lotion an important new addition to the current repertoire of topical moisturizers.

The clinical efficacy of the humectant combination can be attributed to the targeted combination of humectants with physicochemical properties predicted to target the deeper, middle, and surface layers of the SC. This complementary strategy, suggested by modeling, may be further enhanced by synergy among the humectant components, as the efficacy of glycerol is improved when delivered with other humectant molecules,[18] although further investigation into the mechanism underlying this effect is needed.

Continued research into the understanding of humectant properties, mechanisms of skin moisturization, and skin biophysics and biology will undoubtedly generate new insights leading to further advances in moisturizer technology, including the development of highly targeted and more effective functional ingredients.

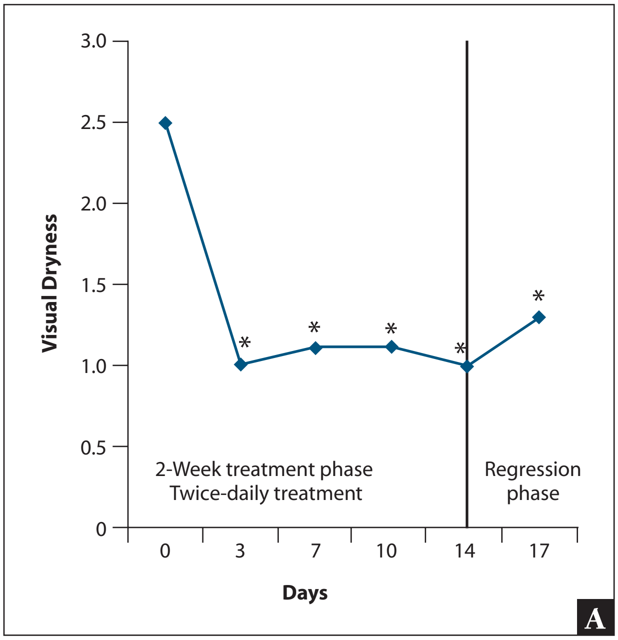

Figure 6. Long-term moisturizer efficacy in winter xerosis. Efficacy of moisturizer containing humectant combination on visual dryness (A) and transepidermal water loss (B) during a 2-week treatment period and subsequent 3-day regression phase with treatment discontinuation. Asterisk indicates $P \leq .05$ compared with baseline.

	Eczema Area and Severity Index Score Total (Arms/Legs Involvement)	*P* Value vs Baseline
Baseline	0.72/0.880	
Week 2	0.680/0.779	.691
Week 4	0.380/0.485	.042

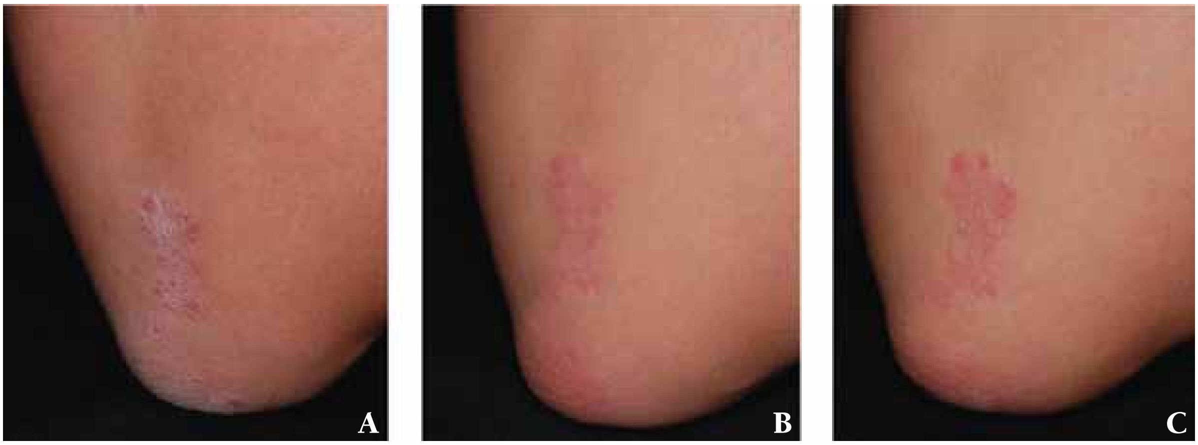

Figure 7. Efficacy of fully formulated humectant mixture as adjunctive moisturizer to eczema treatment. Dermatologist assessment of eczema severity at baseline and during the course of the 4-week study. Photographs of eczematous lesions taken at baseline (A), week 2 (B), and week 4 (C).

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