Original Contribution

Journal of Cosmetic Dermatology, **12**, 247–253

Dyspigmentation, skin physiology, and a novel approach to skin lightening

Zoe Draelos, MD,[1] Amanda Dahl, BS,[2] Margarita Yatskayer, MS,[2] Nannan Chen, PhD,[2] Yevgeniy Krol, BS,[3] & Christian Oresajo, PhD[2]

[1]*Dermatology Consulting Services, High Point, North Carolina, USA*
[2]*L'Oréal Research and Innovation, Clark, New Jersy, USA*
[3]*Skinceuticals, INC., New York, New York, USA*

Summary

Background Even facial pigmentation is considered a universal sign of youth and beauty in all cultures and at all ages in both men and women. The recent FDA concern about the safety of topical hydroquinone has provided the impetus for research into new pigment lightening alternatives in the cosmetic OTC market.
Aim This research examined a novel hydroxyphenoxy propionic acid, ellagic acid, yeast extract, and salicylic acid formulation applied twice daily compared to the standard prescription combination of 4% hydroquinone cream and 0.025% tretinoin cream applied nightly.
Method This single-center investigator-blinded 12 week study enrolled 82 subjects (7 male, 75 female) ages 25–60 years divided into 2 balanced groups of 41 subjects each with one group using a novel hydroxyphenoxy propionic acid, ellagic acid, yeast extract, and salicylic acid formulation applied twice daily compared to the standard prescription combination of 4% hydroquinone cream and 0.025% tretinoin cream applied nightly.
Results Significant tolerability issues arose with the prescription combinations that were not seen with the cosmetic formulation. In terms of ability to even skin tone, decrease spot intensity, decrease spot size, and improve overall pigmentation, both products demonstrated parity.
Conclusion This research demonstrated the value of cosmetic formulations as part of a treatment regimen for pigmentation issues.

Keywords: dyspigmentation, ellagic acid, skin lightening

Introduction

Dyspigmentation of the face is universally undesirable among men and women of all ages, ethnicities, and nationalities transcending Fitzpatrick skin type. An assessment of irregular pigmentation appears to be a hard-wired component of appearance evaluation, and even skin color is a prerequisite for human beauty. Yet, obtaining even skin color is physiologically challenging given environmental conditions, the fragility of the melanocyte, and the effects of aging.[1]

The color of human skin is derived from 3 primary chromophores: melanin, hemoglobin, and collagen.[2] Melanin, the main chromophore, is a skin pigment produced by melanocytes in the basal cell layer of the epidermis found in two forms as eumelanin and pheomelanin. Eumelanin is the ancestral form, while

Correspondence: Z D Draelos, Department of Dermatology, Duke University School of Medicine, Durham, North Carolina, 2444 North Main Street, High Point, NC 27262, USA. E-mail zdraelos@northstate.net

Accepted for publication September 9, 2013

pheomelanin represents a point mutation. Eumelanin is found in two colors, brown and black, while pheomelanin is a red pigment. Pheomelanin accounts for the reddish colors of the lips, areola, and genitalia in all men and women, while eumelanin is responsible for the white to tan to brown to black skin colors, producing the diversity of skin colors found in humans. Eumelanin a polymer of dihydroxyindole, dihydroxyindole carboxylic acid, and reduced forms possesses a spare electron that can be donated to a reactive oxygen species preventing UV-induced damage. Eumelanin becomes oxidized itself when the electron is donated, resulting in darkening of the pigment, accounting for the phenomenon known as the immediate pigment-darkening reaction.[2] This is in contrast to pheomelanin, a polymer of benzothiazine, which is unable to donate an electron, thus making the reddish pigment ineffective in protecting against UV-induced damage.[3] This physiologic reality explains the increased propensity for nonmelanoma skin cancer, lentigines, and photoaging among individuals with pheomelanin predominance. Thus, persons with both eumelanin and pheomelanin production require products that can improve facial dyspigmentation.[4] A commonly used prescription regimen for the treatment for facial dyspigmentation is 4% hydroquinone cream in combination with 0.025% tretinoin cream once nightly. The tretinoin functions as a penetration enhancer for the 4% hydroquinone and also inhibits the transfer of melanin to the melanosomes.[5] The 4% hydroquinone inhibits the production of melanin by interruption of tyrosinase, a key enzymatic step in melanin production. Many patients find use of this combination challenging due to the poor tolerability profile of the tretinoin and the safety concerns regarding hydroquinone raised by the FDA.[6] This recognition led to the search for safe alternatives with superior tolerability and comparable efficacy.[7] A novel formulation containing hydroxyphenoxy propionic acid, ellagic acid, yeast extract, and salicylic acid was developed as a possible alternative. These active ingredients are listed in order of decreasing concentration in the formulation.

Hydroxyphenoxy propionic acid, active ingredient of the highest concentration, decreases melanin synthesis *in vitro*.[8] Melanocytes from the B16 cell line were cultured in fetal calf serum for 3 days with and without hydroxyphenoxy propionic acid. The melanocytes cultured with the ingredient demonstrated less melanin production, but the viability of the cells was not affected. This is an important industry-standard evaluation for the safety of skin-lightening agents as decreased melanin production can occur from melanocytes with decreased viability. This was not the case with hydroxyphenoxy propionic acid, also known as RadianSkin. It is also thought that the hydroxyphenoxy propionic acid inhibits melanin transfer to the keratinocytes. The combination of hydroxyphenoxy propionic acid with ellagic acid, pigment-lightening ingredient of the second highest concentration, pairs a substance inhibiting melanin production with a natural phenol antioxidant found in numerous fruits, including blackberries, cranberries, raspberries, strawberries, and pomegranates.[9] Nondietary sources of ellagic acid include the North American white oak and the medicinal mushroom Phellinus linteus. Plants produce ellagic acid from the hydrolysis of tannins, such as ellagitannin and geraniin. It functions in the plant kingdom as a potent antioxidant and in humans as antioxidant and anti-inflammatory. Ellagic acid inhibits tyrosinase activity and regulates melanin production at the basal epidermal layer by quenching copper ions.[10]

The other two active ingredients in the skin-lightening formulation are a yeast extract and salicylic acid. The yeast extract is derived from saccharomyces cerevisiae and is obtained from the water-soluble constituents of yeast after lysis of yeast and filtering out debris of their membranes. The extract is then dried and ground to powder. It plays a role in the stimulation of lysosomal degradation in keratinocytes. This may help in melanin degradation and removal.[11] The addition of salicylic acid enhances the penetration of the three active ingredients discussed and encourages exfoliation, aiding in the desquamation of pigment containing keratinocytes.

The goal of this study was to evaluate the skin-lightening ability and tolerability profile of a new cosmetic formulation containing hydroxyphenoxy propionic acid, ellagic acid, yeast extract, and salicylic acid compared with the combination of 4% hydroquinone cream and 0.025% tretinoin cream.

Methods

This single-center investigator-blinded study enrolled 82 subjects (seven men and 75 women) aged 25–60 years divided into two balanced groups of 41 subjects each. The groups were balanced for age, severity of dyspigmentation, and Fitzpatrick skin type (35 Fitzpatrick I, 31 Fitzpatrick II, 7 Fitzpatrick III, 8 Fitzpatrick IV, and 1 Fitzpatrick V). Following completion of the informed consent process (Concordia Institutional Review Board, New Jersey) and a past medical history evaluation, subjects were assessed by the dermatologist investigator for appropriateness for study entry. Only subjects with moderate facial dyschromia, defined as a score of 3 on a 5-point ordinal scale, were enrolled,

who also possessed mild-to-moderate fine lines and wrinkles and lack of skin firmness. The facial dyschromias deemed appropriate for inclusion were mottled hyperpigmentation and lentigines, but not melasma. Subjects were required to avoid excess sun exposure and the use of artificial tanning methods. Subjects with other facial dermatological disorders, including active facial acne, or those with a history of hypersensitivity to any of the study product ingredients were excluded. Other exclusion criteria were the use of topical retinoids or cosmeceutical preparations within 2 weeks of study entry and pregnancy or breast-feeding. Subjects who had used other OTC or prescription skin-lightening preparations within 3 months of study entry were excluded. All subjects were required to leave their oral medications unchanged for the duration of the study.

Subjects were randomly assigned to either group 1 or group 2. Half of the subjects were assigned to group 1 and were instructed to use the APC study product (Advanced Pigment Corrector, Skinceuticals Inc., New York, NY, USA) twice daily. The other half was assigned to group 2 and used a generic 4% hydroquinone cream (Perrigo, Allegan, MI, USA) in combination with a generic 0.025% tretinoin cream (Actavis, Parsippany, NJ, USA) once nightly. All study products were masked to blind the subjects as to their identity. The investigator was blinded and removed from the dispensing products. Both groups were provided an identical cleanser for twice daily use and an SPF 30 product for morning application and as needed.

Assessments were performed at baseline, week 4, week 8, and week 12. An ordinal 5-point rating scale was used for all investigator and subject assessments (0 = none, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe). The investigator efficacy assessments included dark spot size, dark spot intensity, hyperpigmentation, visual smoothness, tactile smoothness, skin tone clarity, skin tone evenness, firmness, radiance/brightness, imperfections/blotchiness, and overall facial appearance. The investigator also assessed tolerability on the same 5-point ordinal scale in terms of erythema, edema, dryness, and peeling. The subjects assessed product tolerability on the same 5-point ordinal scale in terms of stinging, tingling, itching, and burning. A noninvasive assessment of corneometry was performed with a single DermaLab pin probe set to collect measurements over 30 s after reaching steady state. The same technician performed all measurements on the left cheek below the zygomatic arch. Digital high-resolution jpeg images of the frontal, right, and left face were also obtained.

The ordinal nonparametric data, including investigator efficacy assessments, investigator tolerability assessments, and subject tolerability assessments, were analyzed using a Mann–Whitney two-tailed *t*-test when normality was established. A t-test was used to evaluate corneometry measurements. Data were assessed longitudinally, evaluating change from baseline for each treatment arm in an intragroup comparison. Intergroup evaluations were also performed as difference from baseline and comparing the raw ordinal ratings for each treatment arm. These multiple evaluation techniques were used as both treatment arms utilized efficacious skin-lightening formulations. Statistical significance level was set at P-value ≤ 0.05.

Subjects were randomized based on their assigned study identification numbers. Study identification numbers were assigned randomly based on the order in which the subjects presented to the research center. This random study product assignment resulted in balanced groups in terms of sex, Fitzpatrick skin color, and severity of dyspigmentation.

Results

Of 82 subjects, 78 completed the research study. Four subjects in group 2 using the generic 0.025% tretinoin and generic 4% hydroquinone cream discontinued due to adverse experiences consisting of extreme facial irritation, redness, stinging, and burning. No subjects in group 1 using the APC study product discontinued.

Corneometry

The noninvasive corneometry data were very interesting as the APC study product did not induce irritation while irritation is a frequently observed side effect with the 0.025% tretinoin cream (RA) and 4% hydroquinone cream (HQ) combination treatment. Indeed, the RA/HQ group did show a statistically significant ($P = 0.006$) increase in corneometry using the difference from baseline analysis technique at week 4. This was probably due to edema induced by RA/HQ irritation that increased the water content of the skin and resulted in increased conductivity and a higher corneometry score than the APC study product. The edema was not visually appreciated by the investigator as part of the tolerability assessments. After week 4, the increase in corneometry was no longer statistically significant in the RA/HQ subjects probably due to facial retinization and adaptation to the irritation. Subjects who are retinoid naïve frequently exhibit this phenomenon. No

statistically significant differences in corneometry were noted between the groups at weeks 8 and 12.

Investigator assessments

The investigator assessments indicated no statistically significant differences between the two groups at baseline, indicating proper balancing of the groups for all assessed parameters and a normal distribution. At week 4, investigator's assessment showed a statistically significant improvement in visual smoothness ($P = 0.018$) and tactile smoothness ($P = 0.013$) for APC group over the RA/HQ group. However, the RA/HQ group experienced some tolerability issues by week 4. The tolerability data for the complete study are presented in Figure 1 with each data point representing the mean ordinal score for each group. Those in the APC group demonstrated significantly less dryness (Fig. 1a), peeling (Fig. 1b), and erythema (Fig. 1c) than those in the RA/HQ group indicating a better investigator-assessed tolerability profile for the APC group.

In terms of pigmentation parameters, APC showed superior improvement in spot size ($P = 0.034$) and imperfections/blotchiness ($P = 0.025$) at week 8. No statistically significant differences were seen at week 12 between the two groups; thus, all of the pigmentation parameters (even skin tone, spot intensity, spot size, hyperpigmentation) and other skin appearance parameters (tactile smoothness, clarity, imperfections, radiance, firmness, overall appearance) demonstrated parity.

The intragroup longitudinal assessment showed a statistically significant improvement in skin visual smoothness ($P = 0.001$) and skin tactile smoothness ($P = 0.001$) at week 4 for the APC group. This translated to a 21% improvement in skin visual smoothness and a 22% improvement in skin tactile smoothness induced by the APC group. This was not seen in the RA/HQ group. However, only APC showed a statistically significant improvement in radiance at week 8 ($P = 0.038$). In addition, only APC group showed statistically significant improvement in imperfections ($P = 0.005$) and firmness ($P = 0.003$) at week 12.

Overall, at week 12, the APC group showed higher percentages of improvement in all parameters than the RA/HQ group, with the exception of dark spot intensity. The investigator-rated percent improvement at week 12 as compared to baseline for both groups is presented in Table 1. In terms of pigmentation, the APC group demonstrated parity with the RA/HQ group and might have performed slightly better in terms of improvement. Also, APC improved pigmentation by 14%, while the RA/HQ group had a 10% improvement in pigmentation. An example of the pigmentation improvement seen in the APC group on the forehead (Fig. 2) and cheek (Fig. 3) after 12 weeks of twice daily application is presented.

Subject tolerability

The subjects were asked to assess the tolerability of their test product in terms of stinging, tingling, itching, and burning. It should be remembered that four subjects using the RA/HQ dropped out of the study due to intense irritation and they discontinued prior to study completion. Thus, their tolerability data were not collected at week 12. The subjects did not note any tolerability differences between the APC and the RA/HQ over the 12 weeks of the study as evaluated by the direct comparison method. The intragroup longitudinal analysis demonstrated a statistically significant increase in itching over baseline ($P = 0.026$) with the RA/HQ group at week 4. No problems were noted with the APC group at any time point.

Summary

This study compared a novel hydroxyphenoxy propionic acid, ellagic acid, yeast extract, and salicylic acid cosmetic formulation applied twice daily, to a combination prescription treatment of 4% hydroquinone cream and 0.025% tretinoin cream applied nightly. All subjects in the study used the same cleanser and sunscreen-containing moisturizer to eliminate the effects of differing photoprotection on the observed pigment lightening. Furthermore, the study was conducted moving from winter to spring eliminating the effect of natural skin lightening due to climatic variation and less natural sun exposure. The study conduct period of this study would have encouraged more pigmentation, which was not seen in either study group. Finally, the sample size of 78 completed subjects is robust based on power calculations, indicating that the findings could not be due to chance alone. These three factors are important considerations in human pigment-lightening research to insure the accuracy of the observations.

The study also enrolled persons of Fitzpatrick skin types I–V, providing insight regarding the ability of the two regimens to function over a broad range of skin pigmentation. It is important to note that all subjects that discontinued were in the RA/HQ arm and were of Fitzpatrick skin type IV. The irritation induced by the RA/HQ caused paradoxical early skin darkening in these individuals and they were discontinued to prevent an

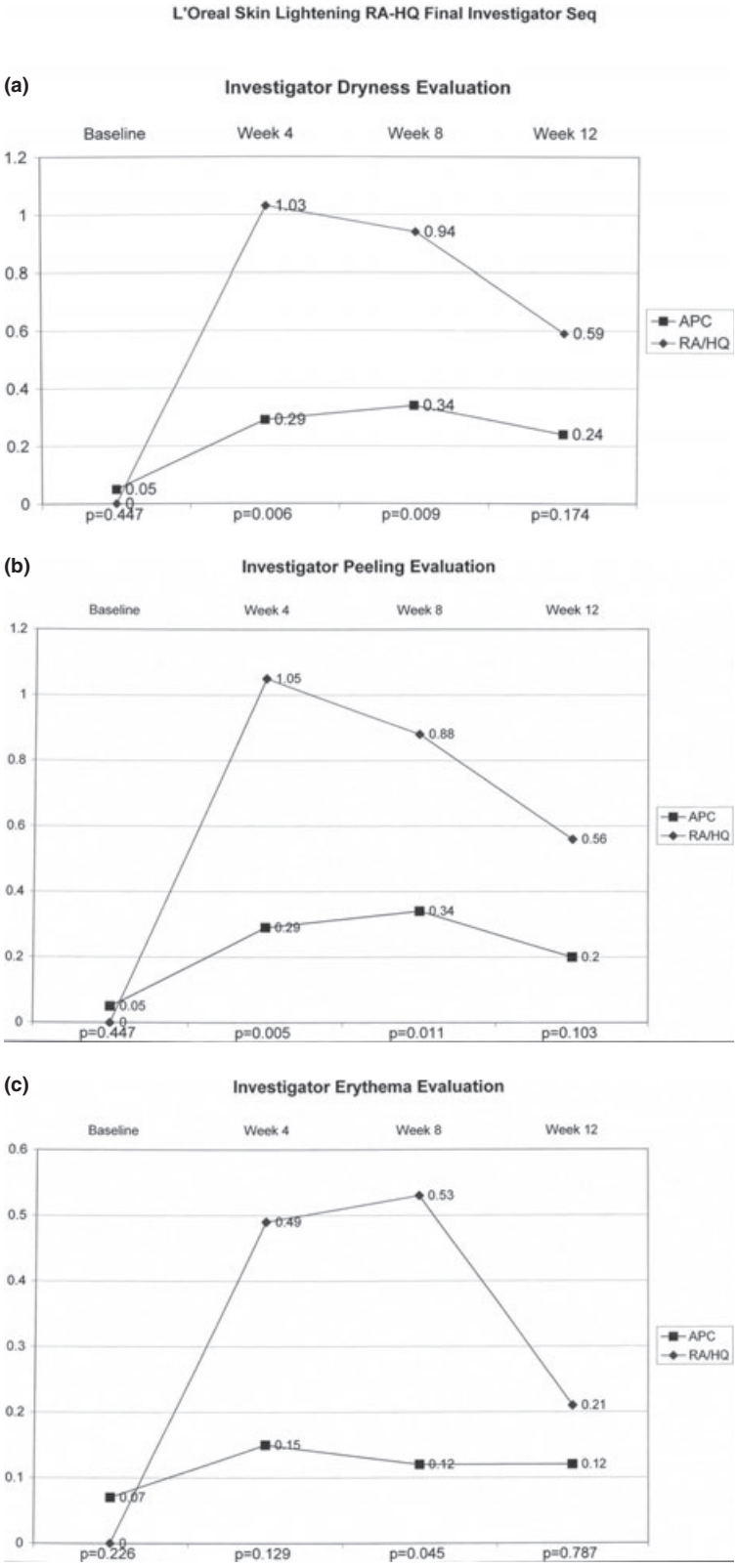

Figure 1 Tolerability comparison between the APC study product and RA/HQ comparator.

anticipated adverse event. This kind of incidence underscores the importance of appropriate formulations for individuals with higher Fitzpatrick skin types. No tolerability issues arose with the cosmetic APC formulation in higher Fitzpatrick skin type individuals.

While hydroquinone can be used as a standalone ingredient in OTC and prescription skin-lightening preparations due to its inhibition of tyrosinase, the rate-limiting step in melanin synthesis, cosmetic formulations usually combine multiple ingredients to obtain the same efficacy. The APC formulation combined hydroxyphenoxy propionic acid to decrease melanin production with ellagic acid which is also known to decrease melanin production and as an anti-inflammatory agent. Yeast extract was added to increase melanin degradation and removal, while salicylic acid enhanced desquamation and penetration. This multi-ingredient approach allowed the cosmetic formulation to reach parity with the prescription RA/HQ formulation in pigment lightening yet provides a superior tolerability profile.

The present study was run for 12 weeks where the APC study produced a 19% decrease in spot intensity, 15% decrease in spot size, and 14% decrease in pigmentation as compared to the RA/HQ combination, which produced a 20% decrease in spot intensity, 13% decrease in spot size, and 10% decrease in pigmentation. These are quite comparable results, yet more pigmentation improvement is desirable and might have been achieved with longer duration use of the study products. At present, a longer duration study is

This research is a step forward in understanding how to formulate and test pigment-lightening cosmetic products. It appears that multiple ingredients utilizing various mechanisms of action can provide similar results to the RA/HQ combination. It is important to use a known pigment-lightening regimen, such as the RA/HQ, to benchmark new cosmetic technology to give the dermatologist a frame of reference for expected efficacy.

References

1 Draelos ZD. Skin lightening preparations and the hydroquinone controversy. *Dermatol Ther* 2007; **20**: 308–13.
2 Angelopoulou E, Understanding the color of human skin. Proc SPIE4299, Human Vision and Electronic Imaging VI 243:June 8, 2001
3 Hill HZ, Hill GJ. UVA, pheomelanin, and the carcinogenesis of melanoma. *Pigment Cell Res* 2000; **13**: 140–4.
4 Halder RM, Richards GM. Management of dyschromias in ethnic skin. *Dermatol Ther* 2004; **17**: 151–7.
5 Torok HM, Jones T, Rich P, Smith S. Hydroquinone 4%, tretinoin 0.05%, flucinolone acetonide 0.01%: a safe and efficacious 12-month treatment for melasma. *Cutis* 2005; **75**: 57–62.
6 McGregor D. Hydroquinone: an evaluation of the human risks from its carcinogenic and mutagenic properties. *Crit Rev Toxicol* 2007; **37**: 887–914.
7 Chang TS. An updated review of tyrosinase inhibitors. *Int J Mol Sci* 2009; **10**: 2440–75.
8 Cognis Patent US8247447 B2 Use of derivatives of 4-hydroxyphenoxy acetic acid.
9 Yoshimura M, Watanabe Y, Kasai K *et al.* Inhibitory effect of an ellagic acid-rich pomegranate extract on tyrosinase activity and ultraviolet-induced pigmentation. *Biosci Biotechnol Biochem* 2005; **69**: 2368–73.
10 Shimogaki H, Tanaka Y, Tamai H, Masuda M. *In vitro* and *in vivo* evaluation of ellagic acid on melanogenesis inhibition. *J Cosmet Sci* 2000; **22**: 291–303.
11 L'Oreal Patent WO 2012072951 A1 Use of an extract of yeast of the saccharomyces genus to improve the brightness of the skin tone, and composition containing at least said extract and a depigmenting agent.