

A CLINICAL STUDY OF TOPICAL PYRATINE 6 FOR IMPROVING THE APPEARANCE OF PHOTODAMAGED SKIN

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Abstract

Objective: Pyratine 6™ has been shown to have antiaging effects in human skin cells. The purpose of this study is to determine the cosmetic efficacy and tolerance of topical Pyratine 6 (0.10%) over 12 weeks for improving the baseline clinical signs and symptoms of photodamaged facial skin.

Methods: A single-arm longitudinal study with observations at 2, 4, 8, and 12 weeks was conducted. Forty healthy women with mild to moderate signs of photodamaged facial skin applied Pyratine 6 twice daily for 12 weeks. Efficacy and safety were evaluated by clinical observations, digital photography, transepidermal water loss (TEWL), skin capacitance, and silicon replicas at each time point.

Results: Topical Pyratine 6 achieved significant improvement from baseline in roughness and skin moisture content after 2 weeks. After 4 weeks, significant improvement in fine wrinkles, mottled hyperpigmentation, and TEWL were observed. Improvements in most parameters were maintained throughout the remaining weeks of the study. For most silicon replica parameters, changes were consistent with increased skin smoothing. Facial erythema was reduced at 2 weeks and further reduced at 4, 8, and 12 weeks. Adverse effects were minimal and transient.

Conclusions: Treatment with Pyratine 6 over 12 weeks improves roughness and skin moisturization in 2 weeks compared to baseline and mottled hyperpigmentation and fine wrinkles in 4 weeks compared to baseline. Reduction in facial erythema occurs as early as 2 weeks. Adverse effects are minimal and transient.

Introduction

Pyratine 6™ has been shown to have modulatory, anti-reactive oxygen species (anti-ROS), and antisenesence effects on the growth of human skin cells.¹ Pyratine 6 (furfurylamino-tetrahydropyran-yladenine) is structurally similar to N6-furfuryladenine except for the addition of the tetrahydropyran-yl group. *In vitro* studies of attachment frequency, cellular growth curves, mitochondria activity, lysosomes, accumulation of intracellular debris, and overall rejuvenation showed Pyratine 6 has antiaging effects that appear to be attributable to the tetrahydropyran-yl group, although its mechanism of action has not been established. The topical formulation of Pyratine 6 has also been shown to increase moisture content in hairless mice.¹

The primary objective of this study is to determine the cosmetic efficacy and tolerance of topical Pyratine 6 (0.10%) for improving the clinical signs and symptoms of photodamaged facial skin over a 12-week period of treatment.

Materials and Methods

This single-arm longitudinal study with repeated observations at 2, 4, 8, and 12 weeks was conducted. Forty healthy women (aged 30 to 65, Fitzpatrick skin types 1-3) with mild to moderate signs of photodamaged facial skin enrolled in an open-label study. The exclusion criteria for participation included pregnancy or lactation; global severity score greater than 6 (10-point scale); history, evidence, or both of chronic

or reoccurring facial skin disease or disorder; and recent use of systemic retinoids; topical retinoids; systemic corticosteroids; topical corticosteroids; topical products containing alpha hydroxy acids, beta hydroxy acids, or both (5% or higher concentration); chemical peels or peptide products; phenol or trichloroacetic acid (TCA) peels; light to medium peels; botulinum toxin type A; or dermal fillers. The study was approved by an institutional review board and signed informed consent was obtained from all patients.

Patients were instructed to wash their faces and apply Pyratine 6 to the entire face in the early morning and approximately 1 hour before bedtime for 12 consecutive weeks from January to April 2006. Patients applied sunblock (SPF 30) in the morning after Pyratine 6 had been absorbed into the skin. Patients were not permitted to use lotions, moisturizers, other skin care products (except products provided by the testing facility), or medications on their faces during the treatment period. Subjects were permitted to use color cosmetics (eg, foundation, blush) without antiaging ingredients. Color digital photographs of the faces were obtained at baseline and at each visit and used as a basis for assessing overall improvement in skin aging at postbaseline time points.

Only patients who completed at least 1 postbaseline visit and complied with the treatment regimen were included in the statistical analysis of cosmetic efficacy data. The analysis was based on the observed data; no data was used from

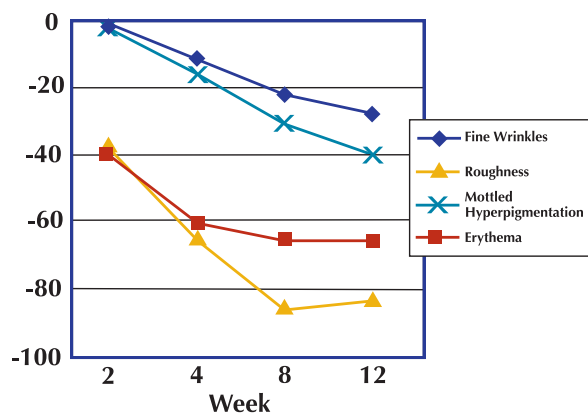
subjects who withdrew from the study prematurely. Patients were assessed for tolerance and improvement in skin aging indicators at weeks 2, 4, 8, and 12. Transepidermal water loss (TEWL) and skin moisture measurements were made on the cheeks of the patients at all visits. Silicone replicas were taken on both canthi and analyzed to document any changes in the appearance of fine lines. Safety was determined by assessments of skin irritation and erythema, and from patient self-assessment diaries.

Digital photographs were obtained with the Fuji S2 Pro camera attached to a VISIA-CR™ Facial Imaging Booth (Canfield Scientific, Fairfield, NJ). Full (global), right, and left facial profiles of each patient were photographed using general purpose (standard), polarized (cross and parallel), and ultraviolet (UV) lighting. Close-ups of left and right periorbital areas were taken with the camera fitted with a dual flash system (Canfield Scientific). Patients were placed in a stereotactic facial positioning device (Canfield Scientific) to ensure exact positioning of the face from one visit to the next.

At each visit, facial fine wrinkles, roughness, and mottled hyperpigmentation were scored using a 5-point scale (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe), and irritation and erythema using a 4-point scale (0=none, 1=mild, 2=moderate, 3=severe). Transepidermal water loss was measured with a Tewameter 300® (Courage-Khazaka, Köln, Germany) in duplicate and skin capacitance was measured with a NOVA DPM 9003® (NOVA Technologies, Gloucester, MA) in triplicate, both after at least 15 minutes of equilibration (70°F ± 3°F; 40% ± 10% relative humidity).

Transepidermal water loss, a noninvasive measurement (g/m²/hr) of water vapor loss through the skin, is a true reflection of stratum corneum barrier function in the absence of sweat. When stratum corneum barrier integrity is improved or damaged, the TEWL decreases or increases,

Figure 1. Inpatient percentage changes in fine wrinkles, skin roughness, mottled hyperpigmentation, and erythema. For fine wrinkles, changes were significant at week 4 ($P=0.004$), week 8 ($P<0.001$), and week 12 ($P<0.001$). For roughness, changes were significant at all time points ($P<0.001$). For mottled hyperpigmentation, changes were significant at week 4 ($P=0.018$), week 8 ($P<0.001$), and week 12 ($P<0.001$). For erythema, reductions from baseline were significant at all time points.

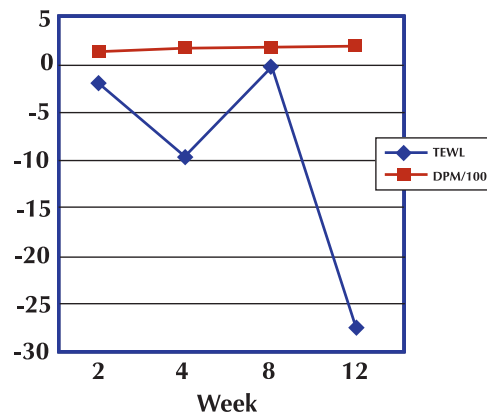


respectively. Skin capacitance is determined by measuring changes in impedance (resistance to alternating current) with skin hydration. As skin becomes more hydrated, impedance decreases and capacitance increases; the higher the skin capacitance, the greater the level of moisture in skin.

Silicone replicas (impressions) of the right and left sides of the periorbital areas were obtained using the Silflo product (CuDerm Corporation, Dallas, TX). Replicas were analyzed by optical profilometry.⁵ Four rough-surface parameters of skin topography were determined: R_z (maximal optical roughness), R_a (average optical roughness), and F_{num} (number of fine line markers), all of which decrease with skin smoothing; and F_{space} (indicator of fine line spacing), which increases with skin smoothing. Replica images were also divided into 10 equal sub-areas to permit detection of shadow-like features and measurement of breadth, shadows, total number of features (eg, wrinkles), and spacing. Breadth, shadows, and the total number of wrinkles decrease with skin smoothing and spacing increases with skin smoothing. At follow-up visits, global improvement of skin was scored relative to a color photograph (full frontal, standard lighting) taken at baseline using a 6-point scale (1=excellent, 2=marked, 3=moderate, 4=slight, 5=none, 6=worse).

Primary efficacy endpoints in this study were the changes, relative to baseline, in skin aging indicators over the 12-week period. Results from the study are presented using descriptive and inferential statistics. The significance of the facial skin changes from baseline for multinomial ordinal variables (wrinkles, roughness, and other parameters in which scores were assigned according to scales) was determined using a Wilcoxon signed rank test at each postbaseline time point. Changes in silicone replica values, TEWL, and skin moisture content are continuous variables and were tested for signifi-

Figure 2. Mean transepidermal water loss (TEWL, g/m²/hr) and dermal phase meter (DPM) values (arbitrary units) on both cheeks. Percentage change was calculated by subtracting the baseline (week 0) TEWL from each posttreatment value, dividing the difference by the baseline value, and multiplying by 100. At each time point, the percentage change was calculated using only data from patients with scores at both baseline and the time point in question. The TEWL for each patient is the average TEWL of the right and left cheeks.



ificance using a paired difference *t* test. Probability values less than 0.05 ($P < 0.05$) were considered significant.

Results

Thirty-four patients completed the study. Five discontinued because they could not make the scheduled visits. One discontinued herself after experiencing dryness for 2 weeks. The dryness resolved completely after 17 days. Changes in skin parameters during the treatment period are summarized in Table 1 and presented graphically in Figures 1 and 2. For Figure 1, changes were calculated by subtracting the mean posttreatment score from the mean baseline score, dividing this difference by the baseline score, and multiplying by 100. Negative values indicated improvement and positive values indicated worsening compared to the baseline. Skin roughness achieved statistically significant reduction at 2 weeks followed by mottled hyperpigmentation and fine wrinkles at 4 weeks (Figure 1). Improvement in these 3 key components of photodamaged skin continued throughout the remaining weeks.

For both TEWL and skin moisturization (Figure 2), the one-factor repeated measures analysis of variance test results showed that the inpatient changes from baseline differed significantly over the 4 post-baseline time points ($P < 0.001$). Results from the Student-Newman-Keuls test revealed that the individual changes were all significantly different from one another in the following rank order: 12 weeks minus baseline response > 8 weeks minus baseline response > 4 weeks minus baseline response > 2 weeks minus baseline response. These results provide clear evidence of an increased beneficial effect with respect to time.

Silicon replicas of fine periorbital wrinkles were made at all visits to obtain objective improvement data. R_z and R_a trended toward lower (nonsignificant) values which suggested increased skin smoothing. The increase in F_{space} was significant at week 8, indicating smoother skin, and the week 12 value trended lower. Spacing trended toward higher (nonsignificant) values, suggesting smoother skin. The decrease in F_{num} at week 8 was significant and trended higher

Figure 3. A 63-year-old woman before a), after 4 weeks b), and after 12 weeks c) of treatment with Pyratine 6 showing reduction in erythema and mottled hyperpigmentation and improvement in fine wrinkles in the right periorbital area.

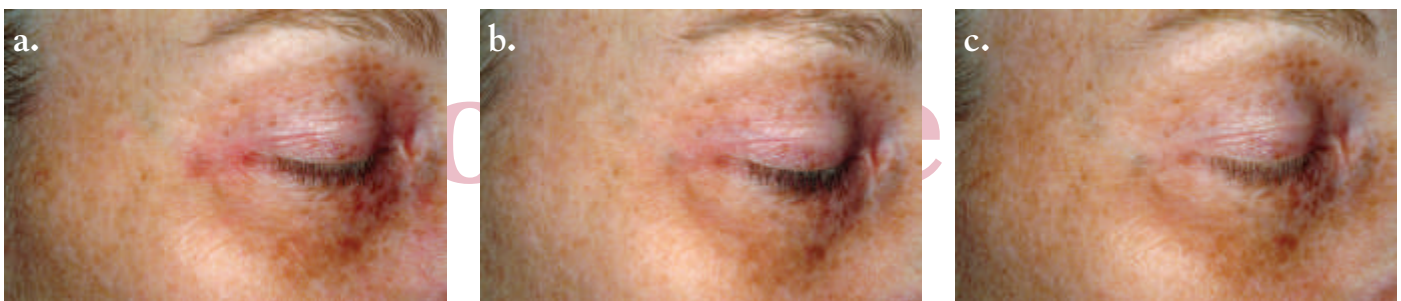


Table 1. Scores, percentage changes from baseline, and times of improvement in skin aging indicators.

Indicator	Week										Improvement	
	0 (Baseline)		2		4		6		8			
	Score	Change (%)	Score	Change (%)	Score	Change (%)	Score	Change (%)	Score	Change (%)	Earliest (Weeks)	Duration (Weeks)
Fine wrinkles*	2.23	0.00	2.18	-1.28	1.97	-11.25 (s)	1.74	-21.79 (s)	1.62	-27.63 (s)	4	4-12
Roughness*	1.68	0.00	1.05	-37.31 (s)	0.58	-63.79 (s)	0.23	-85.71 (s)	0.26	-83.64 (s)	2	2-12
Mottled hyperpigmentation*	1.73	0.00	1.70	-1.45	1.50	-15.63 (s)	1.23	-30.65 (s)	1.03	-40.68 (s)	4	2-12
TEWL	13.15	0.00	12.89	-1.98	11.70	-9.62 (s)	13.0	0.11	9.54	-27.79 (s)	4	4,12
Skin Moisture	118.03	0.00	125.37	6.22 (s)	141.30	20.96 (s)	158.04	35.10 (s)	165.42	41.21 (s)	2	2-12

*From baseline; scale: 0=none; 1=minimal; 2=mild; 3=moderate; 4=severe.

TEWL=Transepidermal water loss.

(s)=Significant difference.

at week 12, indicating smoother skin. The increase in breadth at week 12 and the reductions in number of wrinkles at weeks 8 were significant. The number for wrinkles at week 12 was 1.4% higher than the week 8 value. Changes in breadth were not consistent with skin smoothing whereas the fine wrinkle reductions suggested improvement in skin smoothing. Shadows trended toward lower (nonsignificant) values, consistent with skin smoothing. Clinical examples of improvements in periorbital fine wrinkles are presented in Figure 5.

Treatment with Pyratine 6 was well tolerated by 33 of the 34 patients in this study. Safety was further evaluated by assessments of skin irritation and erythema at 2, 4, 8, and 12 weeks. The mean skin irritation score was 0 at all time points, so values were not calculated or plotted. Compared to baseline, reductions in mean erythema scores (Figure 1) for patients with erythema at baseline were significant at all time points.

All adverse events (AEs) were recorded and evaluated as "possibly related," "probably related," and "definitely related" to the use of Pyratine 6. All AEs were transient and none were serious. Only 1 AE, tingling around the eye ($n=1$), was definitely related. Skin irritation as reported for retinoids and alpha hydroxyl acids^{5,6} was not observed.

Discussion

The data (Table 1) demonstrate that topical Pyratine 6 applied daily for 12 weeks achieves significant improvement from baseline in roughness and skin moisture content after 2 weeks and in fine wrinkles, mottled hyperpigmentation, and TEWL after 4 weeks. Improvements in most parameters were maintained throughout the remaining weeks of the study. Silicon replica data for fine wrinkles indicated improvement in skin smoothness in all parameters except breadth. Significant changes occurred in F_{space} and F_{num} at 8 weeks. An important finding is the significant reduction in erythema at 2 weeks and the additional reductions at weeks 4, 8, and 12. To the authors' knowledge, this is the first report to show reduced erythema associated with use of a topical cosmeceutical product.

Topical formulations of vitamin C² and of growth factors³ for photorejuvenation have been evaluated. Both reports included histological evidence of new collagen formation associated with treatment. The vitamin C study showed improvement in wrinkles, skin texture, and hydration in 1 or more facial areas treated, while the growth factor study showed improvement in wrinkles and skin texture. Improvements were reported only at the end of the study periods, 12 weeks² and 60 days,³ so comparison with the results of our study at 2, 4, and 8 weeks is difficult. This study indicated the advantages

Figure 4. A 63-year-old woman before a), after 4 weeks b), and after 12 weeks c) of treatment with Pyratine 6 showing reduction in erythema and mottled hyperpigmentation and improvement in fine wrinkles in the right periorbital area.

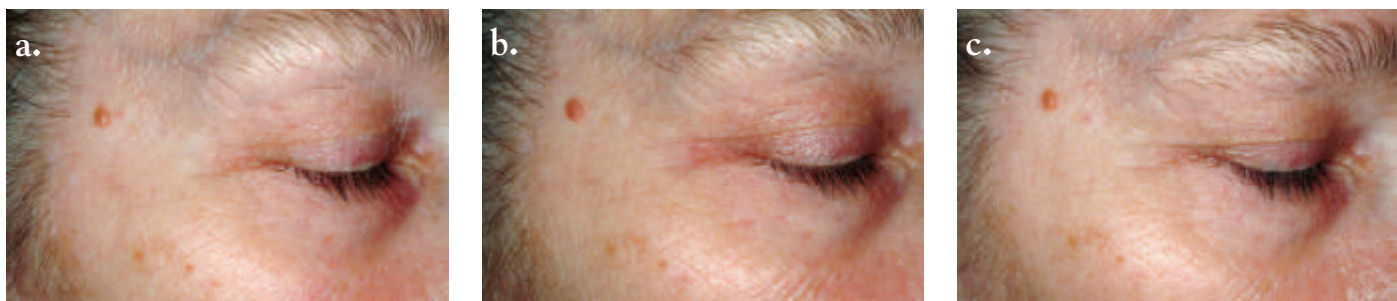
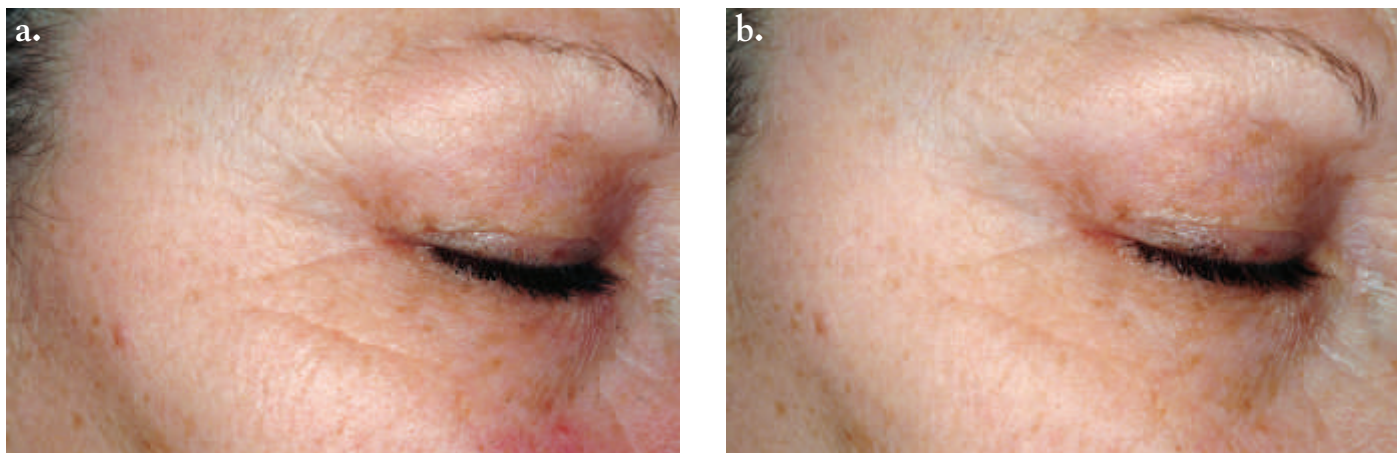


Figure 5. A 54-year-old woman before treatment a) and after 12 weeks of treatment b) with Pyratine 6 showing improvement in fine wrinkles in the right periorbital area.



of Pyratine 6 in the early improvements in roughness, skin moisturization, fine wrinkles, and mottled hyperpigmentation. Neither the vitamin C^{2,4} nor growth factor³ formulations were shown conclusively to improve pigmentation.

Conclusion

Treatment with Pyratine 6 (0.10%) over 12 weeks improves roughness and skin moisturization in 2 weeks and mottled hyperpigmentation and fine wrinkles in 4 weeks compared to baseline. Reduction in facial erythema compared to baseline occurs as early as 2 weeks. Adverse effects are minimal and transient. The encouraging results of this study warrant a multicenter, placebo-controlled clinical trial to further evaluate the clinical benefits of Pyratine 6.

Disclosure

Dr. McCullough is a consultant to Senetek PLC. Dr. Garcia is a consultant to RCTS Inc.

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