Effect of Topical Fluorouracil Cream on Photodamage Secondary Analysis of a Randomized Clinical Trial

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**IMPORTANCE** Photoaging, which is premature skin aging caused by long-term UV exposure, is of aesthetic concern to many patients.

**OBJECTIVE** To investigate the effect of topical fluorouracil, 5%, cream on photoaging using validated photonic numeric scales.

**DESIGN, SETTING, AND PARTICIPANTS** The Veterans Affairs Keratinocyte Carcinoma Chemoprevention Trial was a randomized clinical trial of 932 US veterans with a recent history of 2 or more keratinocyte carcinomas performed from September 30, 2011, through June 30, 2014, to assess the chemopreventive effects of a standard course of topical fluorouracil. Photographs were taken at baseline and at numerous time points for up to 4 years. In our secondary analysis, 2 independent dermatologists graded these photographs using 4 validated photonic numeric scales. A total of 3042 photographs from 281 participants randomized to apply topical fluorouracil or placebo were evaluated at baseline, 6 months, 12 months, and 18 months using 4 photonic numeric scales (Griffiths scale, Allergan forehead lines scale, melomental folds scale, and crow’s feet scale). Data analysis was performed from November 1, 2016, to January 1, 2017.

**INTERVENTIONS** Participants were randomized to apply topical fluorouracil, 5%, cream or a vehicle control cream to the face and ears twice daily for 2 to 4 weeks for a total of 28 to 56 doses.

**MAIN OUTCOMES AND MEASURES** Effect of a standard course of fluorouracil on the extent of photodamage as measured using 4 photonic numeric scales.

**RESULTS** The study population was predominantly male (274 [97.5%]) and white (281 [100%]), with a mean (SD) age of 71.5 (0.57) years. No statistically significant changes were found in photodamage between baseline and 6 months (Griffiths scale: $\chi^2 = 0.01, P = .93$; Allergan forehead lines scale: $\chi^2 = 0.18, P = .67$; melomental fold scale: $\chi^2 = 0.03, P = .87$; crow’s feet scale: $\chi^2 = 2.41, P = .12$), 12 months (Griffiths scale: $\chi^2 = 1.39, P = .24$; Allergan forehead lines scale: $\chi^2 = 0.64, P = .43$; melomental fold scale: $\chi^2 = 0.12, P = .73$; crow’s feet scale: $\chi^2 = 1.07, P = .30$), and 18 months (Griffiths scale: $\chi^2 = 3.11, P = .08$; Allergan forehead lines scale: $\chi^2 = 0.89, P = .34$; melomental fold scale: $\chi^2 = 1.64, P = .20$; crow’s feet scale: $\chi^2 = 0.46, P = .50$).

**CONCLUSIONS AND RELEVANCE** This study did not demonstrate improvement in photoaging with a standard course of topical fluorouracil, 5%, cream, a finding that may be attributable to a true lack of effect in photodamage or limitations of the photonic numeric scales in capturing the effect. The development of photonic numeric scales that include manifestations of photoaging other than rhytids, such as lentigines, hyperpigmentation, and telangiectasias, should be considered.

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photoaging, which is premature skin aging attributable to long-term UV radiation exposure, is of aesthetic concern to many dermatologic patients. Photoaging manifests with signs such as lentigines, rhodies, telangiectasias, inelasticity, and hyperpigmentation.1,2 Numerous modalities, including over-the-counter topicals, topical retinoids, chemical peels, and laser therapy, are used to counteract, prevent, or treat the signs of photoaging.

Normal aging of the skin includes decreased keratinocyte proliferation and differentiation, decreased number of melanocytes, decreased number of fibroblasts, and decreased biosynthesis of collagen.3,4 The mechanism of UV radiation-induced skin change is multifactorial and includes the formation of free radicals along with direct cellular injury.5 In terms of topical agents, retinoids have been the mainstay of therapy for photoaging and functions by activating retinoic acid receptors and retinoid X receptors, 2 families of nuclear receptors that are decreased in the setting of UV radiation.6

Topical fluorouracil, 5%, typically used in the treatment of actinic keratosis, has been suggested for use in reversing photoaging. Improvement in skin texture and wrinkling has been observed with systemic fluorouracil use; however, the effect on skin texture with topical application has not been well studied.7-11 Topical fluorouracil causes epidermal injury, which stimulates dermal remodeling and wound healing.7,12 This epidermal damage stimulates collagen formation, with collagen synthesis verified by molecular measurements.9,12 An analogue of uracil, it is converted into active metabolites that incorporate into RNA and DNA. It inhibits thymidylate synthase by forming a stable complex and preventing the synthesis of deoxythymidinemonophosphate, a key event in DNA replication and repair, with downstream effects of inflammation and apoptosis.8,13 The wound healing pattern demonstrated with fluorouracil use may be similar to that seen in deep chemical peels or laser therapy for photoaging, with up-regulation of keratin 16, interleukin 1β, matrix metalloproteinases, and messenger RNA levels of types I and III procollagen, which have been associated with wound healing and dermal matrix remodeling.12 Specifically, coarse wrinkling, fine wrinkling, tactile roughness, mottled hyperpigmentation, sallowness, and overall global severity improve with topical application of fluorouracil cream.12

Many validated scales have been developed for objective and reproducible comparisons of signs related to photoaging. Both global scales of photoaging and scales to evaluate isolated anatomical locations or specific manifestations of photoaging have been validated. The Griffiths scale is a 9-point global scale that demonstrates the spectrum of photodamage using representative photographs portraying fine wrinkling, coarse wrinkling, mottled hyperpigmentation (including melasma but not including lentigines or nevi), and yellowing (sallowness).14 Validated scales of isolated findings (static horizontal forehead lines, infraorbital hollows, transverse neck lines, facial fine lines, facial skin texture, chin retrusion, and temple volume deficit) have also been developed using morphed images that exhibit the spectrum of aging in the specific area of interest only while keeping the rest of the image unchanged.15-21 Our study sought to investigate change in photodamage in an elderly male population after a standard course of topical fluorouracil, 5%, cream using several standard validated photonic scales.

Methods

VAKCC Trial
From September 30, 2011, through June 30, 2014, the Veterans Affairs Keratinocyte Carcinoma Chemoprevention Trial (VAKCC) enrolled and followed up 932 US veterans across 12 sites with a history of 2 or more keratinocyte carcinomas in the previous 5 years, including 1 on the face or ears. The study sought to assess the chemopreventive effects of a standard course of topical fluorouracil on keratinocyte carcinomas. Participants were randomized to topical fluorouracil, 5%, cream or a vehicle control cream to be applied on the face and ears twice daily for 4 weeks (goal of 56 doses, minimum of 28 doses). Participants presented for visits at baseline, 2 weeks, 4 weeks, 6 months, and every 6 months after up to 48 months. Data on sun protection (sunscreen and hat) use in the past week were collected by survey at baseline and the 6-month visit. The VAKCC Trial was approved by the Veterans Affairs Central Institutional Review Board. The study was conducted in accordance with the Declaration of Helsinki principles.22 Written informed consent was provided by all participants, and all data were deidentified.

Participants were informed of potential cutaneous reactions to the study cream, including erythema, itch, burning, crusting, swelling, and scaling of the skin. These cutaneous reactions were measured by subjective report from the participants at their study visits. In addition, at each visit, photographs of the face and ears were taken at 3 angles (en face, 90° left side of face, and 90° right side of the face).

For the present ad hoc secondary analysis, participants were randomly chosen from 4 randomly selected sites of the VAKCC Trial. Photographs from these participants were evaluated by 2 independent masked dermatologists (K.K., K.C.L.). The time points evaluated were baseline, 6 months, 12 months, and 18 months. For each time point, photographs for all 3 angles were evaluated. Data analysis was performed from November 1, 2016, to January 1, 2017.
Photonumeric Grading

Four validated photonumeric grading scales were used to grade the photos: the Griffiths scale, the Allergan forehead lines scale, a melomental folds scale, and a crow’s feet scale.\(^{14,16,23,24}\) The Griffiths scale is a 9-point visual scale with representative photographs (en face and side profile) for guidance for grades 2, 4, 6, and 8. The Allergan forehead lines scale, melomental scale, and crow’s feet scale are 5-point scales based on isolated anatomical location and specifically evaluate rhytides. En face views of representative photographs are shown for each grade. Scales that involved assessing dynamic photoaging were not used because most photographs graded did not include facial expressions.

One score was assigned to each time point (baseline, 6 months, 12 months, and 18 months) using each scale. Photographs of all 3 angles at any given time point were considered in deciding 1 score; if a discrepancy arose (ie, crow’s feet on the left side and crow’s feet on the right side were different scores), the higher score, representing a higher degree of photoaging, was chosen.

Statistical Analysis

Statistical analysis was conducted using Stata, version 14.0 (StataCorp). For each scale separately, the grades of the 2 raters (K.K., K.C.L.) were averaged. The Kruskal-Wallis test was used to evaluate change in this score between 6 months and baseline, 12 months and baseline, and 18 months and baseline in the placebo vs intervention groups. The Cohen \(\kappa\) coefficient was used to evaluate agreement between the 2 raters, with 0 to 0.20 indicating slight agreement, 0.21 to 0.40 fair agreement, 0.41 to 0.60 moderate agreement, 0.61 to 0.80 substantial agreement, and 0.81 to 1.00 excellent agreement.\(^{25,26}\) The intraclass correlation coefficient was used to determine test-retest reliability.

Results

A total of 3042 photographs from 281 participants were evaluated at baseline (843 photographs), 6 months (747 photographs), and 18 months (714 photographs) using each photonumeric scale. The study population was predominantly male (274 [97.5%]) and white (281 [100%]), with a mean (SD) age of 71.5 (0.57) years. Demographic data of the study population are presented in Table 1.

The Cohen \(\kappa\) coefficient between the 2 raters showed scores ranging from 0.61 to 0.67 for all scales. Test-retest reliability of the raters demonstrated intraclass correlation coefficient values ranging from 0.87 to 0.95. For each scale, no statistically significant changes were found in photodamage between baseline and 6 months (Griffiths scale: \(\chi^2 = 0.81, P = .93\); Allergan forehead lines scale: \(\chi^2 = 0.18, P = .67\); melomental fold scale: \(\chi^2 = 0.03, P = .87\); crow’s feet scale: \(\chi^2 = 2.41, P = .12\)), 12 months (Griffiths scale: \(\chi^2 = 1.39, P = .24\); Allergan forehead lines scale: \(\chi^2 = 1.64, P = .43\); melomental fold scale: \(\chi^2 = 0.12, P = .73\); crow’s feet scale: \(\chi^2 = 1.07, P = .30\)), and 18 months (Griffiths scale: \(\chi^2 = 3.11, P = .08\); Allergan forehead lines scale: \(\chi^2 = 0.89, P = .34\); melomental fold scale: \(\chi^2 = 1.64, P = .20\); crow’s feet scale: \(\chi^2 = 0.46, P = .50\) (Table 2). At the 6-month visit, the incidence of sunscreen or hat use in the past week was 80%.

Discussion

A standard course of topical fluorouracil, 5%, cream did not result in improvement of photodamage that could be appreciated with photographs using 4 validated photonumeric scales. Studying the effect of topical fluorouracil on photoaging is important because photoaging is of significant concern to a large population. Many patients who are prescribed topical fluorouracil cream for actinic keratoses are hesitant to use it because of its cutaneous adverse effects (including cosmetic adverse effects, such as redness). Demonstrating improvement in photoaging may encourage these patients to consider its use.

Previously, 2 prospective studies\(^{7,14}\) examined the effect of topical fluorouracil on photoaging, showing signs of improvement in a small sample size. Sachs et al\(^{12}\) evaluated 21 healthy volunteers, of whom 19 completed the study by applying fluorouracil twice daily for 2 weeks. Investigators evaluated baseline and 6-month photographs using the Griffiths scale and took biopsy specimens from the treated areas. They also evaluated wrinkling, tactile roughness, lentigines, hyperpigmentation, and sallowness in-person visits. They noted an improvement in the Griffiths scale, as well as course wrinkling, fine wrinkling, hyperpigmentation, lentigines, and sallowness. Furthermore, biopsies investigating the molecular response to fluorouracil treatment revealed an increase in effectors of epidermal injury (keratin 16), inflammation (interleukin 1β), extracellular matrix degradation (matrix metalloproteinases 1 and 3), and increased type I procollagen protein levels.\(^{12}\)

Similarly, Guimarães et al\(^{7}\) investigated the effect of topical fluorouracil on the forearms in a photodamaged population. In a prospective trial of 32 patients, significant clinical and cosmetic improvement was noted in photoaging in terms of wrinkles, lentigines, and actinic keratoses and in manifestations of dermatoporosis (skin fragility from aging and long-term sun exposure), such as purpura. Biopsy specimens of the forearms taken 1 month after initiation of treatment were sent...
for immunohistochemical analysis, revealing decreased dermal elastosis and reduced levels of p53 with increased levels of procollagen I.

Our study suggests that topical fluorouracil does not produce a noticeable effect on photoaging in an elderly male population with photoaging. Although it is possible that a standard course of the medication has no effect on overall photoaging, histologic and immunohistochemical effects in the studies described previously suggest that there is such an effect. However, both studies used a small sample size and did not provide a control group for comparison. This finding raises the concern that the photonumeric scales used in our study were not sensitive enough to measure the effect of a standard course of treatment with topical fluorouracil. Specifically, 3 of 4 of our scales evaluated only rhytides. The scales may not be able to capture the aspects of aging affected by fluorouracil. Alternatively, it may suggest that changes produced by fluorouracil are not sustained through our follow-up points of 6 to 18 months or that the study population may exhibit sun damage too severe for differences to be accurately measured with these scales.

The mechanism of topical fluorouracil as previously described involves inhibiting DNA replication and repair, which helps explain efficacy of fluorouracil in treating or preventing actinic keratosis and its potential chemopreventive effects studied in the VAKCC Trial. Although improvement in actinic damage with fluorouracil is known, the effect of fluorouracil on wrinkling, telangiectasias, mottled hyperpigmentation, and other manifestations of photoaging have not been extensively studied. However, the 2 previous small-scale studies noted improvement in these domains.

Our study specifically used the Griffiths global scale, Allergan forehead lines scale, a melomental folds scale, and a crow’s feet scale. These 3 scales specifically capture changes related to static lines. Small changes in fine lines are more difficult to capture with these scales. Although the Griffiths global scale can help account for additional aging factors other than wrinkles, it can still be difficult to adequately use this scale to evaluate the severely photodamaged face, which was common in our population. Previous studies demonstrating improvement with fluorouracil also separately evaluated for lentigines, hyperpigmentation, vascular components, and sallowness, which were factors that were difficult to capture on the scales used in this study. Although the Griffiths scale attempts to capture the global view of all these components, individuals with more lentigines but less vascularity and less volume loss would still be ranked high because of the lentigines component. Looking at each component individually allows for more fine-tuning of these markers of photoaging. A lack of validated scales specific to measuring these components was the primary reason why these factors were not measured in our study.

### Strengths and Limitations

The primary strength of our study is a large sample size in a masked randomized clinical trial of participants with severe sun damage. This large sample size enabled assessment of photodamage changes attributable to fluorouracil alone without confounding factors. Although photodamage improvement with topical fluorouracil has been previously demonstrated, the sample size of that study was significantly smaller (21 patients). Furthermore, the follow-up time period for the study by Guimarães et al was only 1 month, which does not answer the question of whether the effects seen by fluorouracil are sustained over time. Our photographs showed 3 angles of the face with follow-up through 18 months, allowing for a more comprehensive assessment of photodamage and a long-term assessment of the effects of fluorouracil.

Limitations of the study include a homogenous population (primarily elderly and male), which restricts generalizability, and using photographs instead of live patients. It is impossible to depict the 3-dimensional quality of live patients in photographs, which may affect assessment of various aspects of photoaging. For the melomental folds scale specifically, many photographs could not be graded because of facial hair. Finally, although most participants (control and treatment groups) used sunscreen or a hat at least once in the week before 6-month follow-up, more detailed data on regular sunscreen use are not available. Knowledge of the use of regular sun protection practices could affect the interpretation of our findings.

### Conclusions

We were unable to demonstrate improvement in photoaging with a standard course of topical fluorouracil, 5%, cream. Development of photonumeric scales that capture other manifestations of photoaging, such as lentigines, hyperpigmentation, and telangiectasias, should be considered.
Effect of Topical Fluorouracil Cream on Photodamage


