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An *in vivo* model for postinflammatory hyperpigmentation: an analysis of histological, spectroscopic, colorimetric and clinical traits

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Summary

Background Acne vulgaris is a common condition that occurs in all skin types. Postinflammatory hyperpigmentation (PIH) is often associated with acne in patients of darker skin types, making it a common complaint in dermatology offices. Despite this, there is limited understanding of and effective treatment options for PIH.
Objectives The study objective was to validate an *in vivo* model for PIH and to compare the clinical, histological and spectroscopic characteristics of artificially induced PIH and acne-induced PIH.
Methods A nonblinded, nonrandomized pilot study was performed. Thirty subjects served as their own control in which four sites treated with 35% trichloroacetic acid (TCA) solution and four truncal acne pustules were followed for 8 weeks and were evaluated clinically and histologically, and by colorimetry and spectroscopy.
Results The initial phases of inflammation between TCA- and acne-induced PIH differ. However, clinical evaluations were similar on and after day 14. Acne- and TCA-induced lesions were clinically, histologically and spectroscopically indistinguishable at day 28.
Conclusions Clinical, spectroscopic and histological similarities of acne-induced and TCA-induced PIH at day 28 suggest that TCA-induced PIH can be a reproducible model for the study of acne-induced PIH.

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Conflicts of interest
I.H.H. is an investigator for Estée Lauder, Ferndale Laboratories and Clinuvel. H.W.L. is a consultant for Ferndale, Uriage, Sanofi and Johnson & Johnson. He has grant support from Estée Lauder, Ferndale Laboratories and Clinuvel. P.I. is a subinvestigator for Estée Lauder, Ferndale Laboratories and Clinuvel. I.K. is a subinvestigator for Estée Lauder and Ferndale Laboratories. O.N.A. is a subinvestigator for Estée Lauder and Clinuvel. M.S.M. is a full-time employee of Estée Lauder. All other authors have nothing to declare.

This paper was previously presented as an oral presentation at the Skin of Color Society Symposium in Miami, FL, U.S.A. in February 2013 and in Denver, CO, U.S.A. in March 2014; and also as a poster presentation at the Society of Investigative Dermatology in Edinburgh, U.K. in May 2013.

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What's already known about this topic?

- Postinflammatory hyperpigmentation (PIH) is skin discoloration due to multiple insults and more frequently affects darker-skinned patients.
- However, we don't know if different causes of PIH have similar clinical and spectroscopic properties.

What does this study add?

- A model to induce PIH has been introduced.
- It possesses similar clinical, spectroscopic and histological properties as acne-induced PIH. This model will allow us to design future structured mechanistic studies to help manage PIH.

Postinflammatory hyperpigmentation (PIH) is an acquired hypermelanosis. This process can occur in all skin types but more frequently affects darker-skinned patients.[1] PIH can occur after an infection, contact dermatitis, drug reaction, burns, surgical or cosmetic procedures, or any other cutaneous inflammatory insult.[1–4] In people of colour, PIH frequently occurs in resolving acne lesions and can persist for months after the acne lesion itself has disappeared.[5] In many cases, the resulting PIH can be more distressing than the original inflammatory insult.[6] The pathogenesis of PIH includes an increase in melanin production and/or an abnormal distribution of this melanin.[7]

This pilot study was designed to test the hypothesis that PIH caused by iatrogenically induced injury would follow a similar process as acne-induced PIH, hence potentially serving as a model to study the latter. The acute phase of these two inflammatory models would necessarily differ. There are challenges in developing these protocols because there is no validated measure to quantify changes in individual lesions of PIH. Thus, the project focused on comparing the Investigator's Global Assessment (IGA) scale, histology and spectroscopy.

Materials and methods

Patient selection

The Institutional Review Board of Henry Ford Hospital approved this study (IRB #7013). All guidelines from the Declaration of Helsinki were followed. Written informed consent was obtained from all patients. Subjects who were 18 years or older were eligible if they had existing truncal acne with at least four clinically apparent pustules in coexistence with PIH or postinflammatory erythema which were secondary to truncal acne as confirmed by IGA.

Study design

The study was a nonblinded, nonrandomized study. Subjects were recruited at the Department of Dermatology of Henry Ford Hospital, Detroit, Michigan. In subjects meeting the enrolment criteria, four PIH lesions were induced on the right buttock with a 35% trichloroacetic acid (TCA) solution placed onto the skin using a thin cotton swab for 30 s or until the skin showed signs of frosting. The size of each of the four TCA-induced PIH lesions was approximately 0·50 cm^2. Both truncal acne pustules and induced TCA lesions were monitored using diffuse reflectance spectroscopy (DRS), colorimetry, clinical photographs and IGA on days 0, 1, 7, 14, 28, 42 and 56.

Assessments

Clinical global assessment

A six-point IGA scale for hyperpigmentation and erythema (Table 1) was developed by investigators of this study at the Department of Dermatology, Henry Ford Hospital. The scale looks at the contrast between diseased and normal skin. The contrast is what we believe most affects a subject's quality of life and self-esteem and not the overall degree of pigmentation. An IGA of zero corresponds to a subject's normal skin at baseline, and subsequent IGA scores are relative to that baseline. Normalizing against a subject's baseline helps to eliminate any effect of skin phototype on pigmentation assessment.

Colorimetric and spectroscopic measurements

Skin pigmentation was objectively measured with colorimetry, hyperspectral imaging (HSI) and DRS. The colorimeter consisted of a spectrophotometer (Konica Minolta CM-2600d, Osaka, Japan) that used a xenon arc lamp and a computer. The HSI system consisted of a uniform illumination source, Lowel Vip Pro-light halogen tungsten lamp (Lowel-Light Manufacturing, Inc., Hauppauge, NY, U.S.A.) and Lite panel LED panels, a hyperspectral camera with sensor Philumina-VNIR/400H (PhiLumina, LLC, Gulfport, MS, U.S.A.) capable of detecting absorbance in the wavelength range of 400–1000 nm, and a computer. The components of the DRS were the light source (HL-2000 Ocean Optics deuterium tungsten halogen lamp), a broadband spectrophotometer (USB 2000 light detector, BWTEK Inc., Newark, DE, U.S.A.) that was capable of detecting absorbance in the wavelength range 350–850 nm, a 2·5-mm bifurcated fibre-optic probe that was applied to the subject's skin, and a computer. The reflectance spectrometer unit emits visible light onto the skin and then analyses the collected light that is reflected from the skin.

Histological evaluation

TCA was applied on day 0. At day 1, subjects underwent a 4-mm punch biopsy of a representative truncal acne lesion, and

Table 1 Investigator's Global Assessment (IGA) description of hyperpigmentation and erythema

IGA scale	Hyperpigmentation	Erythema
0	Clear of hyperpigmentation	Clear of erythema
1	Almost clear of hyperpigmentation	Almost clear of erythema
2	Mild, but noticeable hyperpigmentation	Mild, but noticeable erythema
3	Moderate hyperpigmentation (medium brown in quality)	Moderate erythema (pink in quality)
4	Severe hyperpigmentation (dark brown in quality)	Severe erythema (dark pink in quality)
5	Very severe hyperpigmentation (very dark brown, almost black in quality)	Very severe erythema (very dark pink, almost red in quality)

of one of the TCA-induced lesions. A second set of biopsies were done of a TCA-induced PIH lesion and of an acne-induced PIH lesion on day 28. Therefore, a total of five skin biopsies were obtained: two from acne lesions (days 1 and 28), two from TCA-induced lesions (days 1 and 28), and one from normal uninvolved skin for the same patient at the end of the study. Samples were stained with haematoxylin and eosin and Melan-A.

Statistical analysis

Statistical analysis was performed to compare the IGA scores of both types of lesions. Comparisons were made of the number of melanocytes and melanophages between normal skin and acne-induced PIH and between normal skin and TCA-induced PIH. Acne-induced PIH and TCA-induced PIH melanocyte and melanophage counts were also compared. Melanin content measured by HSI was compared between normal skin and acne-induced PIH, normal skin and TCA-induced PIH, as well as between acne-induced PIH and TCA-induced PIH. Comparisons were made using a two-tailed paired-sample *t*-test. In cases where distributional normality was significantly violated, the nonparametric Wilcoxon signed-rank test was used instead due to the paired nature of the data. Statistical significance was set at $P < 0{\cdot}05$, and all analyses were done using SAS software (version 9·2, SAS Institute Inc., Cary, NC, U.S.A.).

Results

Study population

Thirty subjects were enrolled and all completed the study. The final data analysis for IGA was done on all 30 subjects. The mean age was 32·6 years (range 18–63). Two subjects were of Fitzpatrick skin type II, five subjects were of skin type IV, 19 subjects were of skin type V, and four subjects were of skin type VI.

Clinical global assessment

Photographs of acne and TCA can be seen in Figures 1 and 2, respectively. IGA scores for hyperpigmentation and erythema over time are shown in Figure 3. In Figure 3a, the high TCA hyperpigmentation IGA, seen on day 1, was a false positive as it was due to necrosis secondary to frosting from the TCA. In day 28 there is a downward trend and subsequent plateauing of the TCA hyperpigmentation IGA, which reflects true hyperpigmentation. In contrast, acne hyperpigmentation trends upward then plateaus at day 28 and then is found to have a similar course as the TCA hyperpigmentation. In Figure 3b, the initial peak in the IGA erythema score for the TCA was also a false positive, secondary to initial inflammatory response to TCA application.

The IGA scores for the acne-induced PIH and TCA-induced PIH were statistically significantly different on days 0, 1 and 7 ($P < 0{\cdot}05$). However, there were no statistically significant differences in the IGA scores for the two lesions on days 14, 28, 42 and 56 ($P > 0{\cdot}05$). Subsequently, the average IGA scores for both acne-induced PIH and TCA-induced PIH were indistinguishable at week 2 and reached a plateau at day 28 with average scores of 2·9 and 3·0, respectively. Over time, IGA scores for acne- and TCA-induced postinflammatory erythema have the same course.

Colorimetric and spectroscopic measurements

Colorimetry and DRS measurements were obtained for all sites for every subject. The data on day 28 were selected for statistical analyses for both these instruments as the IGA scores for acne and TCA were similar at this time point. Colorimetry measurements are expressed as L* (lightness to darkness), a* (green to red) and b* (blue to yellow) colour parameters. DRS measurements are used to determine relative melanin, oxyhaemoglobin and deoxyhaemoglobin content. DRS has been shown to be a good assessment tool for melanin.[8] Therefore, only melanin content was considered for the DRS data analysis. Average acne measurements were determined for each patient per visit. The data was normalized based on their average acne value of that visit. A common statistical method known as discriminant function analysis was used to analyse the spectroscopic measurements.[9] The discriminant function analysis method classifies the data into the independent groups (acne or TCA) by minimizing the variations within the groups and maximizing variations between the groups. The compar-

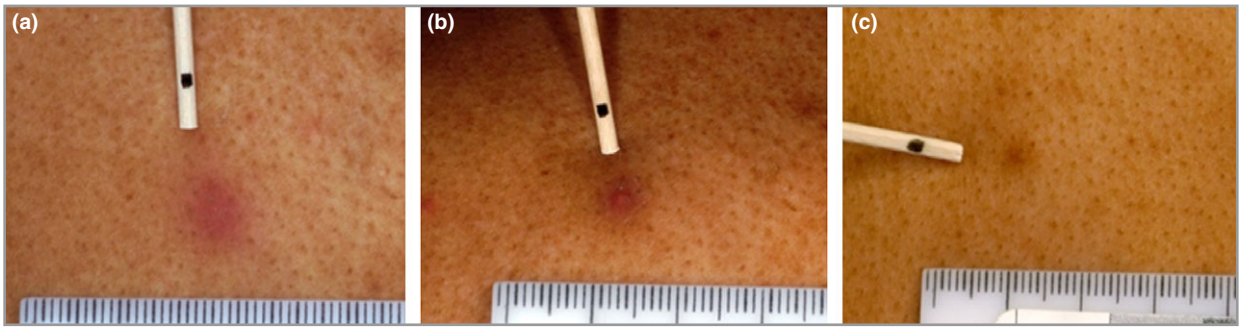

Fig 1. Acne lesion: Investigator's Global Assessment. (a) Day 1: erythema, 4; hyperpigmentation, 0. (b) Day 28: erythema, 3; hyperpigmentation, 2. (c) Day 56: erythema, 0; hyperpigmentation, 3.

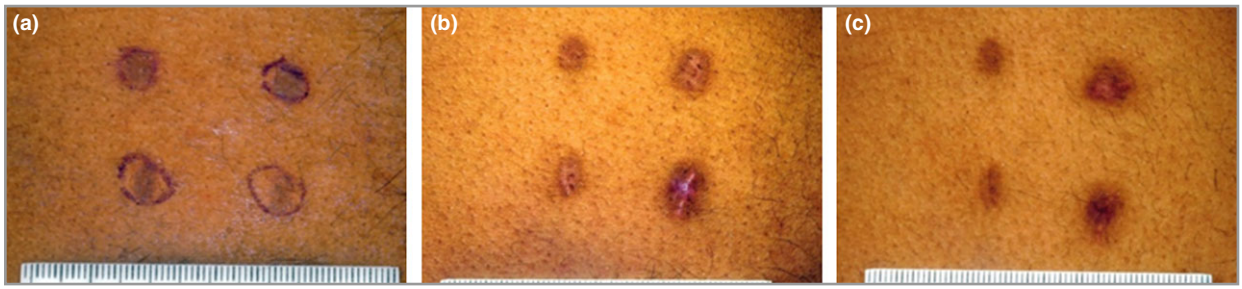

Fig 2. Trichloroacetic acid lesion: Investigator's Global Assessment. (a) Day 1: erythema, 0. (b) Day 28: erythema, 2; hyperpigmentation, 4. (c) Day 56: erythema, 2; hyperpigmentation, 4.

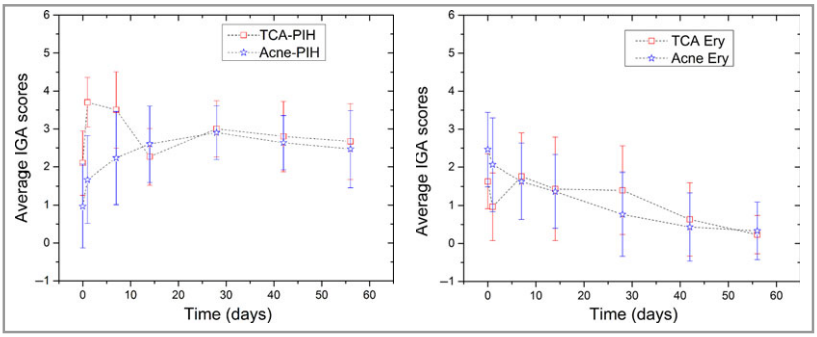

Fig 3. (a) Means and standard errors for IGA scores for hyperpigmentation over time. (b) Means and standard errors for IGA scores for erythema over time. Ery, erythema; IGA, Investigator's Global Assessment; PIH, postinflammatory hyperpigmentation; TCA, trichloroacetic acid.

ison between the two groups (acne and TCA) resulted in only one discriminant function.

The plots of discriminant function against each category for both the colorimetry and DRS data can be seen in Figure 4. Each symbol in the plot represents a single optical measurement. The discriminant scores (*y*-axis) of acne and TCA are very similar and they highly overlap with each other. A leave-one-out cross-validation classification also was performed using their discriminant scores.[10] In this method, each data point is treated as an unknown case and is tested against all the other data points. The classification results were used to determine the sensitivity and specificity of the data. In our analysis, sensitivity [= TP/(TP + FN)] and specificity [= TN/(TN + FP)] were derived based on the following interpretation: true positives (TP) indicate a TCA-induced PIH lesion was correctly identified as a TCA-induced lesion. True negatives (TN) indicate an acne-induced PIH measurement was correctly classified as acne. False positives (FP) indicate an acne-induced PIH measurement was incorrectly classified as a TCA-induced lesion. False negatives (FN) indicate a TCA-induced lesion measurement was incorrectly classified as acne. In other words, the sensitivity is defined as the ratio of true TCA identification over all the TCA data points, and the specificity is determined by true acne identification over the total acne data points. The results showed that the sensitivity of TCA was 51% in colorimetry and 55·3% in DRS. The specificities are 77·5% and 63·8% for colorimetry and DRS, respectively. The sensitivity towards TCA was further reduced to 46·7% and 53·8% for colorimetry and DRS, respectively, when each patient was considered as a test case (one patient at a time) while the rest of the data set was treated as the training set. Specificity values showed no discernible differences (colorimetry 77·6% and DRS 63·3%). The results demonstrate that colorimetry and DRS do not distinguish between TCA-induced PIH and acne-induced PIH and indicate that both share similar optical characteristics.

HSI was done for all sites for 12 subjects on their day 28 visit. HSI measurements were used to determine melanin concentration[11,12] and data was normalized against adjacent normal skin. Table 2 shows the measured melanin content in acne-induced PIH and TCA-induced PIH along with that in adjacent normal skin. There was a statistically significant difference ($P < 0·05$) between melanin content in acne-induced PIH and adjacent normal skin as well as in TCA-induced PIH and adjacent normal skin on day 28. However, there was no statistically significant difference between melanin content in acne-induced PIH and TCA-induced PIH on day 28, indicating the similarity in the optical characteristics exhibited by both lesions. HSI results are consistent with colorimetry and DRS findings.

Histological evaluation

Skin biopsies of normal uninvolved skin, acne-induced PIH and TCA-induced PIH taken on day 28 were analysed. On day 1, acne pustules were characterized by perifollicular neutrophilic inflammation, whereas TCA lesions had full-thickness epidermal necrosis with perivascular and/or perifollicular lym-

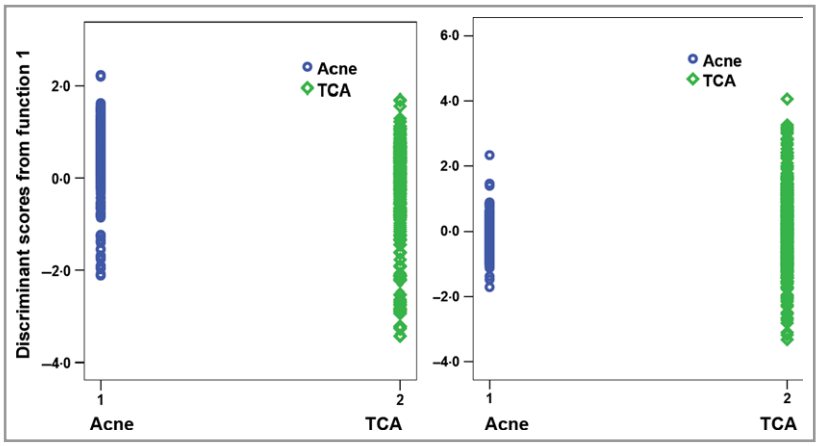

Fig 4. Left: discriminant function plot for colorimetry. Right: discriminant function plot for diffuse reflectance spectroscopy. TCA, trichloroacetic acid.

phocytic inflammation. Skin biopsies obtained on day 1 were excluded during analysis as the majority of TCA samples were found to have evidence of full-thickness epidermal necrosis, thus making it difficult to compare with the acne samples from day 1.

By day 28, both acne-induced PIH and TCA-induced PIH showed perifollicular and perivascular lymphocytic inflammation and dermal fibrosis. Melanophage density was determined by counting the melanophages per 4 high-power fields, while the melanocyte count was done by counting the melanocytes per 0·5 mm of the epidermis. All counts were done, in a blinded fashion, by the same dermatopathologist (M.C.). A comparison of melanophages and melanocytes was performed for normal skin, acne-induced PIH and TCA-induced PIH on day 28, and there were no statistically significant differences from normal skin. Table 3 contains the comparison of melanocytes and melanophages at day 28 between acne-induced PIH and TCA-induced PIH, and is also shown in Figure 5. The mean and median melanocyte number was slightly higher in the TCA-induced PIH samples, but the difference is not statistically significant. For melanophages, the mean is higher for the acne-induced PIH samples, but the difference is also not statistically significant.

Table 2 Melanin content measured in acne-induced PIH and TCA-induced PIH and adjacent normal skin from hyperspectral imaging for 12 patients

Average acne melanin	Average control melanin for acne site (back)	Average TCA melanin	Average control melanin for TCA site (buttock)
0·056467	0·025585803	0·0436	0·02723
0·215752	0·164950029	0·2340	0·18096
0·043785	0·04100552	0·1225	0·08526
0·107896	0·062999554	0·1738	0·13790
0·101724	0·057498571	0·1080	0·06651
0·322422	0·205447582	0·2504	0·25449
0·123757	0·087189432	0·2448	0·18445
0·309831	0·256709902	0·3038	0·26917
0·154633	0·112066221	0·3082	0·25804
0·113893	0·063197817	0·1664	0·14521
0·115667	0·089087433	0·1633	0·09928
0·229533	0·184161474	0·2383	0·21827

PIH, postinflammatory hyperpigmentation; TCA, trichloroacetic acid.

Discussion

To our knowledge, this is the first study to create and validate an *in vivo* model for PIH. In this study, PIH was induced using 35% TCA. This medium-depth chemical peel can cause damage to the epidermis and papillary dermis, thus producing epidermal necrosis, papillary dermal oedema and homogenization, and a sparse lymphocytic infiltrate within the first couple of days.[13] These effects were seen histologically when the TCA lesions were biopsied on day 1 (24 h after TCA application). Because necrosis was the cause of the initial apparent discoloration that was seen in the TCA lesions, they were not given a day 1 IGA score as this was not true hyperpigmentation. Within 7–14 days after applying the TCA, the necrotic crust peeled away and hyperpigmentation subsequently developed, allowing for the IGA scoring to take place on subsequent visits. The IGA for TCA and acne erythema were noted to decrease with time. The IGA for TCA-induced PIH data is also consistent with general clinical observations about acne-induced PIH, in that resolution takes weeks to months.[1,5]

Spectroscopy is a noninvasive method routinely used to objectively evaluate pigmentary disorders.[14,15] The spectroscopic data (colorimetry, DRS and HSI) obtained in this study demonstrate that PIH induced from acne and TCA share similar optical characteristics, validating this as a model to study PIH. Melanin is essential for skin pigmentation.[16,17] The histology results in this study indicated that there was no statistically significant difference in the mean number of counted melanocytes and melanophages in the acne-induced PIH and TCA-induced PIH lesions. Also, no statistically significant difference in the mean number of counted melanocytes and melanophages was reported between acne-induced PIH and normal skin or TCA-induced PIH and normal skin. Because the IGA scores were relative to normal skin and were supported by spectroscopic measurements, it appears that spectroscopy and IGA scores allow for better distinction of PIH from nor-

Table 3 Melanocytes and melanophages at day 28 from histology

	n	Mean (SD)	Median (Min, Max)	P-value
Melanocytes				
Acne	24	8·8 (5·7)	8 (0, 21)	0·408
TCA	24	10·3 (5·7)	10 (2, 21)	
Melanophages				
Acne	29	6·6 (4·8)	5 (0, 15)	0·994
TCA	29	6·4 (4·7)	5 (0, 16)	

MAX, maximum; Min, minimum; TCA, trichloroacetic acid.

mal skin. This supports the fact that spectroscopy and IGA can more effectively distinguish PIH from normal skin than haematoxylin and eosin staining. Additionally, the differences in cellular infiltrate did not appear to distinguish TCA-induced PIH from acne-induced PIH.

Our study was limited by a small sample size and a discrepancy in the number of skin biopsies analysed as some patients refused skin biopsies of normal skin as well as day 28 biopsies. Another limitation was the use of a relatively high concentration of TCA. Although we used 35% TCA, commonly used in a medium-depth chemical peel, patients developed significant necrosis within the first few days, thus making it difficult to assign IGA scores during the initial visits. But, in using 35% TCA we did get consistent pigmentation in darker skin types with slight crusting that results in pigmentation of a similar character to acne-induced pigmentation. Our next study, to evaluate the use of lower TCA concentrations that would induce PIH, should help to look into the development of pigmentation from subnecrotic challenges.

HSI was performed on only 12 subjects due to limited availability of instrumentation and staff. Our final limitation was the use of our nonvalidated IGA scoring system to evaluate the hyperpigmentation and erythema. After the completion of our study, a validated scoring system to measure PIH from acne vulgaris, called the postacne hyperpigmentation index (PAHPI), was reported.[18] The use of the PAHPI in future studies would allow for a more standardized outcome measure as well as assessment of the effectiveness of PIH treatments.

This study did show that spectroscopy and IGA are able to quantify the degree of pigmentation. This further confirms what has been found in previous PIH and melasma studies, where histology was found to be ineffective, but spectroscopy was effective in quantifying pigmented skin from normal skin.[19,20] Thus, the correlation between clinical and spectroscopic evaluation suggests that these two components can be part of future clinical research to assess the efficacy of various interventions done to treat or prevent PIH.

In summary, PIH, which commonly occurs in patients with Fitzpatrick skin type II and above, can lead to psychological stress and negatively impact the quality of life. Currently, there are no uniformly effective treatments for PIH. The results of this pilot study suggest that TCA-induced PIH could be a reproducible model for acne-induced PIH. This initial model for PIH using TCA serves as a foundation for us to better understand as well as improve our ability to manage PIH.

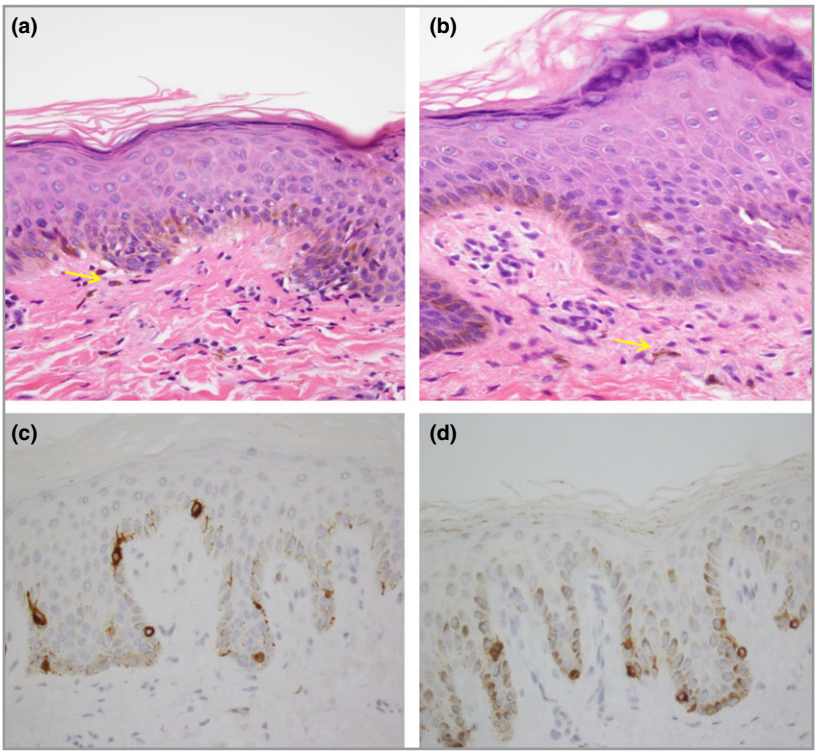

Fig 5. Histological changes at day 28. (a) Acne-induced PIH; (b) TCA-induced PIH. Haematoxylin and eosin, original magnification ×400; arrows pointing at melanophages. (c) Acne-induced PIH; (d) TCA-induced PIH. Melan A stained ×400. PIH, postinflammatory hyperpigmentation; TCA, trichloroacetic acid.

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