Subclinical sensitization with diphenylcyclopropenone is sufficient for the treatment of alopecia areata: Retrospective analysis of 159 cases

Sung Jay Choe, MD, Solam Lee, MD, Long Quan Pi, PhD, Dong In Keum, MD, Chung Hyeok Lee, MD, Beom Jun Kim, MD, and Won-Soo Lee, MD, PhD
Wonju, Republic of Korea

See related letter on page 599

Background: Contact immunotherapy with diphenylcyclopropenone (DPCP) is presently considered the treatment of choice for extensive alopecia areata. However, a major concern with contact immunotherapy is that it causes various adverse effects (AEs) that contribute to discontinuation of treatment.

Objective: We investigated whether a modified DPCP treatment protocol can promote hair regrowth with fewer AEs.

Methods: All patients were sensitized with 0.1% DPCP and began treatment with 0.01% DPCP. Thereafter, the DPCP concentration was slowly increased according to the treatment response and AEs. This was a retrospective review of DPCP treatment with modified protocols in 159 patients with alopecia areata.

Results: Of the 159 patients, 46 (28.9%) showed a complete response and 59 (37.1%) showed a partial response. No patients had AEs after sensitization. During the treatment, only 3 patients (1.9%) showed severe AEs, and 55 showed moderate AEs; however, all were well controlled with antihistamines alone or antihistamines and medium-potency topical steroids. There was no association between treatment response and AEs.

Limitations: Sample size, subject composition, and the retrospective study design represent potential limitations.

Conclusion: A modified DPCP treatment protocol with subclinical sensitization could induce a favorable therapeutic response and result in fewer AEs. (J Am Acad Dermatol 2018;78:515-21.)

Key words: adverse effect; alopecia areata; diphenylcyclopropenone; DPCP; immunotherapy; sensitization.

Alopecia areata (AA) is a chronic inflammatory skin disease characterized by sudden-onset nonscarring hair loss.[1] Various treatment modalities have been used to treat AA.[2-6] Contact immunotherapy was first introduced in 1978.[7] Since then, the efficacy of diphenylcyclopropenone (DPCP) has been evaluated.[8-17] However, the major problem with contact immunotherapy is that it causes various adverse effects (AEs) such as eczema, blistering, and lymph node enlargement that contribute to patient discontinuation of treatment. To address this problem, some reports have shown

Abbreviations used:

AA:	alopecia areata
AE:	adverse effects
CR:	complete response
DPCP:	diphenylcyclopropenone
SADBE:	squaric acid dibutylester

From the Department of Dermatology and Institute of Hair and Cosmetic Medicine, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea.
Funding sources: None.
Conflicts of interest: None disclosed.
Accepted for publication October 29, 2017.
Reprints not available from the authors.

Correspondence to: Won-Soo Lee, MD, PhD, Department of Dermatology, Yonsei University Wonju College of Medicine, 20 Ilsan-ro, Wonju 26426, Republic of Korea. E-mail: leewonsoo@yonsei.ac.kr.
Published online November 3, 2017.
0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2017.10.042

that low-dose treatment without an eczematous reaction could produce a comparable effect at the clinical practice and cellular levels.[18,19] Furthermore, modified squaric acid dibutylester (SADBE) immunotherapy without sensitization was recently reported to be clinically comparable to conventional therapy, with fewer AEs.[20,21]

In our clinic, we treated 5 patients using a split-scalp model after sensitization with 0.1% DPCP as a preliminary study. Of the 5 patients, 3 showed hair regrowth on the right side after treatment of the right side alone, and regrowth on the entire scalp was observed when treatment with the same concentration was extended to the entire scalp, (Fig 1). Of the 5 patients, 3 had no AEs and 2 had mild itching that responded well to antihistamines.

On the basis of these results, we hypothesized that subclinical sensitization with DPCP could result in a sufficient therapeutic response and fewer AEs. Thus, we used a modified DPCP protocol consisting of treatment with 0.01% DPCP after 0.1% DPCP sensitization, even without clinical symptoms, and adjusted the concentration according to treatment response. We aimed to investigate whether this modified protocol promotes regrowth, reduces the number and severity of AEs, and decreases relapse rates.

CAPSULE SUMMARY

- Conventional diphenylcyclopropenone contact immunotherapy has been used in the treatment of extensive alopecia areata but can be associated with severe adverse effects.
- Even without an eczematous reaction after sensitization, sufficient therapeutic responses were achieved without severe adverse effects.
- Sensitization to induce an eczematous reaction may not be required for successful contact immunotherapy.

MATERIALS AND METHODS

Patient selection and demographics

The cases of 159 patients with AA who were treated with the modified DPCP protocol in Wonju Severance Christian Hospital between January 2003 and December 2016 were retrospectively reviewed.

All types of AA, including patchy alopecia, alopecia totalis, and alopecia universalis, were included. Each medical record was reviewed for demographic and clinical information including age, sex, clinical subtype, age at disease onset, disease duration before DPCP treatment, antinuclear antibody titer, medical history, family history of AA, and severity at first visit. Treatment duration, highest DPCP concentration, and AEs were evaluated. This study was approved by the institutional review board of Yonsei University Wonju College of Medicine (CR317059).

Modified protocol of DPCP contact immunotherapy

Treatment was started at the hospital with 0.1% DPCP sensitization. A 2 × 2-cm^2 area or the largest affected patch on each patient's scalp was sensitized. One week after sensitization, lesions on the scalp were treated with 0.01% DPCP. The concentration of DPCP solution was slowly increased (to 0.01%, 0.025%, 0.05%, and 0.1%) depending on the patient's response and clinical course.

We maintained the DPCP concentration when hair regrowth was observed at the application site or when AEs such as pruritus, erythema, and eczema were observed. However, we increased the DPCP concentration if clinical improvement or an eczematous reaction was not observed after 8 treatments. Patients visited the hospital once a week, and the treatment interval was adjusted to once every 2 or 4 weeks depending on the treatment response. Patients underwent DPCP treatment to the entire scalp, including lesions that previously displayed hair loss, even if the lesions had improved. Patients were instructed to avoid direct sun exposure of the treated areas.

Evaluation of treatment response

Clinical hair regrowth responses were assessed at each visit. We used the Severity of Alopecia Score,[22] which is described in the Supplemental Materials and Methods (available at http://www.jaad.org).

Evaluation and management of AEs

After treatment with DPCP, patients were evaluated for the presence of pruritus and for whether lesions and the periphery of the treated site showed erythema, scaling, vesicles, or regional lymph node enlargement. To manage the AEs, antihistamines and/or topical steroids were prescribed according to symptoms and severity. This is discussed in the Supplemental Materials and Methods.

Scalp biopsy and immunohistochemical staining

A skin biopsy was performed to investigate whether DPCP treatment after subclinical

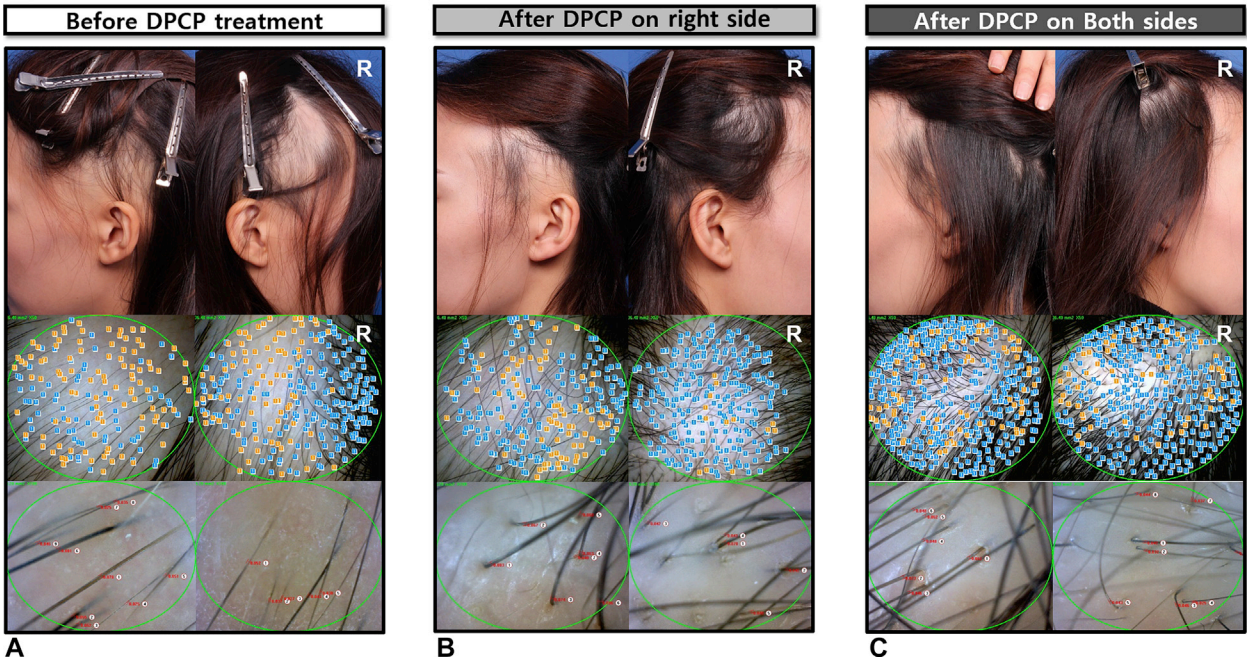


Fig 1. Representative results of sensitization with 0.1% diphenylcyclopropenone (DPCP) and treatment with 0.01% DPCP. **A**, A 27-year-old woman presented with a 10-year history of alopecia areata. After sensitization of the patient to 0.1% DPCP, to confirm the therapeutic efficacy of DPCP immunotherapy, only the right side of the scalp was treated with 0.01% DPCP. **B,** After initiation of treatment, new terminal hair growth was observed on the right side of the scalp after 4 treatments. **C,** As the treated right side improved, both sides were treated with 0.01% DPCP from the seventh treatment. After that, new terminal hair began to grow on the entire scalp, and sufficient clinical improvement was observed without adverse effects during treatment. Clinical photographs (*upper panels*) and phototrichograms were used to follow the overall and local changes of lesions according to DPCP treatment. Phototrichograms were used to determine hair counts (*middle panels*, with terminal hair indicated by *blue dots* and vellus hair indicated by *yellow dots*) and hair thickness (*lower panels*) at each point.

sensitization resulted in an immune response. Five patients in whom AA was initially diagnosed provided informed consent to participate. Specimens were taken from the lesions before DPCP sensitization. Thereafter, the lesions were sensitized to 0.1% DPCP and then treated once a week for 3 weeks with 0.01% DPCP. A follow-up biopsy was performed on the 3 patients in whom hair regrowth had started without any AEs, including pruritus, erythema, and eczema. Immunohistochemical staining was performed to assess the expression of CD1a, CD3, CD4, and CD8 (see Supplemental Materials and Methods).

Statistical analyses

All data were analyzed with SPSS software (version 23.0, IBM, Armonk, NY). Logistic regression analysis was used to analyze the differences in treatment responses according to disease- and treatment-associated factors. Multivariate regression analysis of the statistically significant univariate parameters was performed using a *P* value less than .20 as the initial entry criterion. Differences in relapse rate according to continuation of DPCP treatment were assessed by the chi-square and Kaplan-Meier methods. A *P* value less than .05 was considered statistically significant.

RESULTS

Subject demographics

The demographic and clinical data are shown in Table I.

Absence of AEs in the sensitization process and overall fewer severe AEs during the treatment

None of the 159 patients complained of AEs after sensitization (Table II). During treatment, 61 reported no symptoms, including itching. Itching was mild in 40 patients, moderate in 38, and severe in 20. An antihistamine was prescribed for patients with moderate or severe itching. Of 20 patients with treatment site erythema, 11 showed scaling and were prescribed a topical steroid to manage

Table I. Subject demographics and disease- and treatment-associated factors

Characteristic	Value
No. of patients	159
Sex, n (%)	
Male/female	93 (58.5)/66 (41.5)
Age at visit, y	
Mean	39.8
Range	13-74
Alopecia type, n (%)	
Patchy	114 (71.7)
Alopecia totalis	7 (4.4)
Alopecia universalis	38 (23.9)
Age at onset, y	
Mean	37.4
Range	9-73
Disease duration before DPCP treatment, mo	
Mean	21.2
Range	1-195
ANA abnormality, n (%)	
Within/>normal range	144 (90.6)/15 (9.4)
Autoimmune disease history, n (%)	
Without/with autoimmune disease history	154 (96.9)/5 (3.1)
Atopic dermatitis history, n (%)	
Without/with atopic dermatitis history	151 (95.0)/8 (5.0)
Family history, n (%)	
Without/with family history	152 (95.6)/7 (4.4)
Severity at first visit, n (%)	
Total (75%-100%)	35 (22.0)
Severe (50%-74%)	38 (23.9)
Moderate (25%-49%)	35 (22.0)
Mild (0%-24%)	51 (32.1)
Treatment duration, mo	
Mean	24.3
Range	4-70
Highest DPCP treatment concentration, n (%)	
0.01%	56 (35.2)
0.025%	61 (38.4)
0.05%	28 (17.6)
0.1%	14 (8.9)
Treatment response, n (%)	
Complete response (>90% hair regrowth)	46 (28.9%)
Partial response (50%-90% hair regrowth)	59 (37.1%)
Inadequate to no response (<50% hair regrowth)	54 (34.0%)

ANA, Antinuclear antibody; *DPCP*, diphenylcyclopropenone.

discomfort. Two patients with blisters and 1 patient with lymph node enlargement were also prescribed an antihistamine and a topical steroid; their symptoms of discomfort and skin lesions were well controlled.

Table II. Frequency of AEs and related medication history (N = 159)

Characteristic	Value
AEs after sensitization, n (%)	
None	159 (100)
Itching or rash	0 (0)
AEs after treatment, n (%)	
None	61 (38.4)
Mild itching (PVAS score 1-3)	40 (25.2)
Moderate itching (PVAS score 4-7)	38 (23.9)
Severe itching (PVAS score 8-10)	20 (12.6)
Increased rash	20 (12.6)
Increased scaling	11 (6.9)
Vesicles	2 (1.3)
Lymph node enlargement	1 (0.6)
Medication because of AEs (n = 58), n (%)	
po antihistamine only	47 (81.0)
Topical steroid + po antihistamine	11 (19.0)
Systemic steroid	0 (0)

AE, Adverse event; *po*, by mouth; *PVAS*, Pruritus Visual Analog Scale.

Presence or absence of AE does not affect therapeutic response

We investigated several variables that may affect the treatment response (Supplemental Table I; available at http://www.jaad.org). In logistic univariate analysis, hair loss at sites other than the scalp, longer disease duration before DPCP treatment, and greater extent of scalp lesions at the first visit were associated with a worse treatment response.

No significant difference in treatment response was detected between patients with and without AEs. Furthermore, there was no significant difference in treatment response between patients who did or did not receive medication for AEs.

Four features of AA were significant prognostic factors on univariate analysis and were further evaluated on multivariate analysis. In multivariate analysis, we confirmed that the presence of lesions at sites other than the scalp and high severity at the first visit were correlated with a poor treatment outcome. However, disease duration before DPCP treatment and highest DPCP concentration were excluded from the factors affecting treatment response (P = .211 and P = .124, respectively).

Continued treatment is a strong prognostic factor of decreased relapse rates in patients with AA

Of the 159 patients who received DPCP treatment, 46 who showed a complete response (CR) (>90% regrowth) were evaluated for relapse (Supplemental Table II; available at http://www.jaad.org). Of these,

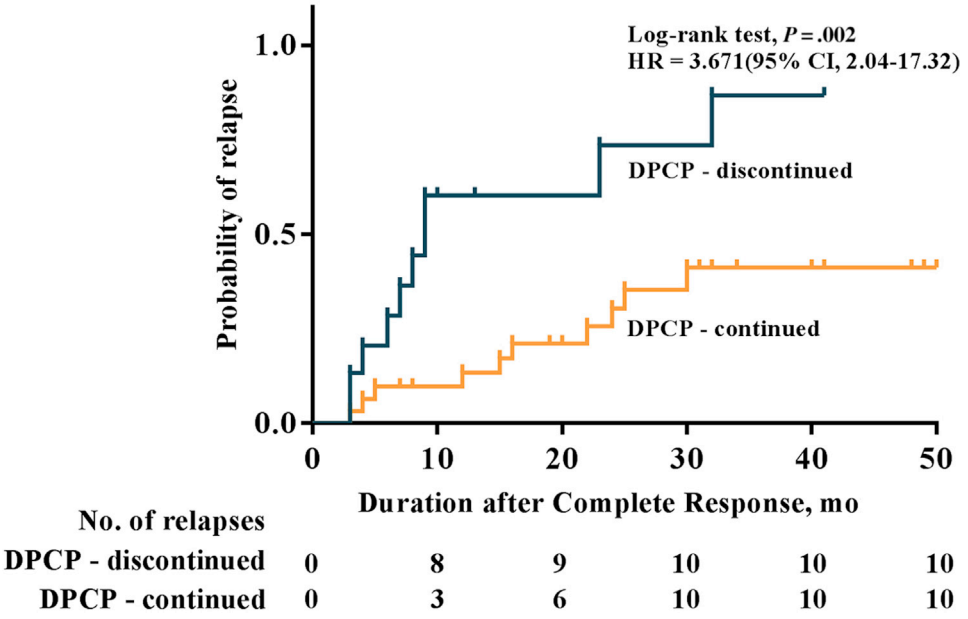


Fig 2. Overall relapse rate analysis using the Kaplan-Meier method. Relapse was compared in patients with alopecia areata (n = 31) and without continuous treatment (n = 15). Patients who underwent continued treatment after achieving a complete response had a longer duration of clinical improvement without relapse. *CI*, Confidence interval; *HR*, hazard ratio.

31 continued treatment and 15 discontinued treatment after achieving a CR. In patients who continued DPCP treatment, the entire scalp was treated at the same concentration used to achieve a CR.

A total of 20 patients (43.5%) relapsed. Of these, 10 continued DPCP treatment. The patients who continued treatment showed a significantly lower relapse rate than those who discontinued treatment. The 46 patients who showed a CR were followed for up to 48 months. On the basis of these findings, the cumulative hazard for relapse was evaluated. Those who discontinued DPCP treatment had a 3.7-fold higher risk for relapse than those who continued treatment (Fig 2).

Subclinical sensitization and 4 DPCP treatments triggered an immune response without clinical symptoms

Before DPCP sensitization, miniaturization and perifollicular and peribulbar lymphocytic infiltration were observed in hair follicles, whereas mild perivascular lymphocytic infiltration was observed in the papillary dermis (Supplemental Fig 1; available at http://www.jaad.org). On immunohistochemical staining, the hair follicles were mainly infiltrated by $CD4^+$ T cells, although $CD8^+$ and $CD1a^+$ T cells were also observed. After 3 DPCP treatments, lymphocytic infiltration was mainly concentrated in the epidermis and papillary dermis. Infiltration by $CD1a^+$, $CD3^+$, and $CD8^+$ T cells was confirmed on immunohistochemical staining. This finding suggests that delayed hypersensitivity could be induced by DPCP even without clinical symptoms. However, the lymphocytic infiltration around the hair follicles varied. Two patients showed decreased perifollicular lymphocyte infiltration; there were no significant changes in cellular infiltration in 1 patient.

DISCUSSION

Several studies established the therapeutic effect of DPCP in patients with AA, but the reported treatment responses have varied.[8-17,23,24] The differences in the response rate depend not only on disease-associated factors but also on treatment-associated factors such as sensitization protocol and treatment area, duration, and interval.[25-29] Furthermore, there are conflicting reports as to whether the presence or absence of an eczematous reaction after initial sensitization affects the therapeutic response. At a low concentration of DPCP that did not elicit an allergic response, Hull et al[19] reported stimulation of hair follicles in AA and Botham et al[18] demonstrated that Langerhans cell counts were increased in presensitized mouse epidermis. Two recent studies reported that treatment with SADBE, even without sensitization, results in treatment success similar to that with conventional treatments.[20,21]

Our study achieved a clinical response (>50% regrowth) rate of 66.0%, including rates of 28.9% in patients with a CR and 37.1% in those with a partial response (50%-90% regrowth). We did not compare these results with those of a control group treated with a conventional protocol. However, the fact that (1) the mean treatment response was 53.75 ± 0.79% in a recent systematic review,[23] (2) a large number of patients participated in this study, and (3) patients were observed for a relatively long period suggests that the modified protocol has therapeutic efficacy comparable to that of a conventional protocol.

Various AEs have been reported in previous studies; however, it is difficult to accurately compare the incidence because of differences in the items described in each study. Thus, we examined the incidence of severe pruritus and blisters caused by severe allergic reactions described in previous studies.[8-17] Severe pruritus was reported at frequencies of 14.0% to 36.7%, whereas 12.6% of patients showed severe pruritus in our study. Blisters were reported at frequencies of 22% to 45.3%. However, very few patients in our study reported blisters. Most patients who experienced AEs could be managed with an antihistamine alone or an antihistamine plus a topical steroid. These findings imply that AEs are less frequent and relatively mild after the use of the modified protocol.

We evaluated the relapse rate of patients who achieved a CR. As already described, the follow-up periods differed among studies, making direct comparisons difficult. However, our results are similar to those of previous studies in terms of patients who continued or discontinued treatment.[8-17] Considering that we followed our patients for up to 50 months (with a mean observation period of 19.6 months), we concluded that our modified DPCP protocol was comparable to that of a conventional protocol.

We also analyzed the factors that could affect treatment outcomes; univariate analysis showed a strong correlation between treatment response and the initial extent of hair loss and body hair involvement, which are well-known prognostic factors.[15] However, treatment response was not correlated with age at onset or family history.[8] The results of multivariate analysis showed that initial extent of scalp hair loss and body hair involvement were also statistically significant prognostic factors for treatment response. This result suggested that the patient composition of our study was representative of the wider population.

However, there was no difference in treatment response according to presence, type, or severity of AEs. In addition, there were no significant differences in treatment response according to DPCP concentration. These findings provide indirect evidence that eczematous reactions during sensitization and treatment are unnecessary.

One likely explanation for these findings is that initiating treatment in the absence of clinically sufficient sensitization lowers the incidence of severe AEs (including eczema) that make it difficult for patients to continue treatment. Therefore, we could apply DPCP to a wider area than in conventional treatment, which is usually applied only to the lesion. Nonlesional skin in AA differs from normal-appearing scalp skin, and a pathogenic immune response has already occurred in the hair follicle before the AA lesion becomes clinically apparent.[30-34] Thus, the findings following treatment of nonlesional as well as lesional skin indicate that subclinical stages of AA can be treated with DPCP and that DPCP application may decrease the likelihood of flares of the condition. In fact, we thought that this explanation was more convincing because patients who continued DPCP treatment even after a CR had a lower relapse rate than did those who discontinued treatment.

Histologic findings before sensitization showed that $CD4^+$ T cells infiltrated the skin around hair follicles. However, follow-up biopsies after sensitization and treatment showed that $CD8^+$ T cells had infiltrated the epidermis and papillary dermis, although the patient showed no eczematous reaction, including pruritus or erythema. As recruited $CD8^+$ T cells in contact allergy contribute to hair regrowth,[21,35-37] we concluded that the intended contact allergic response was induced with our modified DPCP protocol.

This study had limitations in that it did not include a control group of patients treated with the conventional DPCP protocol and only Korean patients participated in this study. Nonetheless, this study suggested that contact immunotherapy after subclinical sensitization has positive therapeutic effects with a lower incidence of AEs. Furthermore, these advantages help the patient continue treatment, thereby reducing relapse rates.

REFERENCES

1. Lee NR, Kim BK, Yoon NY, Lee SY, Ahn SY, Lee WS. Differences in comorbidity profiles between early-onset and late-onset alopecia areata patients: a retrospective study of 871 Korean patients. *Ann Dermatol*. 2014;26:722-726.
2. Jun M, Lee NR, Lee WS. Efficacy and safety of superficial cryotherapy for alopecia areata: a retrospective, comprehensive review of 353 cases over 22 years. *J Dermatol*. 2017;44:386-393.
3. Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part II. Treatment. *J Am Acad Dermatol*. 2010;62:191-202 [quiz 3-4].

4. Hordinsky M, Donati A. Alopecia areata: an evidence-based treatment update. *Am J Clin Dermatol*. 2014;15:231-246.
5. Shapiro J. Dermatologic therapy: alopecia areata update. *Dermatol Ther*. 2011;24:301.
6. Messenger AG, McKillop J, Farrant P, McDonagh AJ, Sladden M. British Association of Dermatologists' guidelines for the management of alopecia areata 2012. *Br J Dermatol*. 2012;166:916-926.
7. Happle R, Cebulla K, Echternacht-Happle K. Dinitrochlorobenzene therapy for alopecia areata. *Arch Dermatol*. 1978;114:1629-1631.
8. Chiang KS, Mesinkovska NA, Piliang MP, Bergfeld WF. Clinical efficacy of diphenylcyclopropenone in alopecia areata: retrospective data analysis of 50 patients. *J Investig Dermatol Symp Proc*. 2015;17:50-55.
9. Luk NM, Chiu LS, Lee KC, et al. Efficacy and safety of diphenylcyclopropenone among Chinese patients with steroid resistant and extensive alopecia areata. *J Eur Acad Dermatol Venereol*. 2013;27:e400-e405.
10. Ohlmeier MC, Traupe H, Luger TA, Bohm M. Topical immunotherapy with diphenylcyclopropenone of patients with alopecia areata—a large retrospective study on 142 patients with a self-controlled design. *J Eur Acad Dermatol Venereol*. 2012;26:503-507.
11. El-Zawahry BM, Bassiouny DA, Khella A, Zaki NS. Five-year experience in the treatment of alopecia areata with DPC. *J Eur Acad Dermatol Venereol*. 2010;24:264-269.
12. Sotiriadis D, Patsatsi A, Lazaridou E, Kastanis A, Vakirlis E, Chrysomallis F. Topical immunotherapy with diphenylcyclopropenone in the treatment of chronic extensive alopecia areata. *Clin Exp Dermatol*. 2007;32:48-51.
13. Aghaei S. Topical immunotherapy of severe alopecia areata with diphenylcyclopropenone (DPCP): experience in an Iranian population. *BMC Dermatol*. 2005;5:6.
14. Cotellessa C, Peris K, Caracciolo E, Mordenti C, Chimenti S. The use of topical diphenylcyclopropenone for the treatment of extensive alopecia areata. *J Am Acad Dermatol*. 2001;44:73-76.
15. Wiseman MC, Shapiro J, MacDonald N, Lui H. Predictive model for immunotherapy of alopecia areata with diphencyprone. *Arch Dermatol*. 2001;137:1063-1068.
16. Gordon PM, Aldrige RD, McVittie E, Hunter JA. Topical diphencyprone for alopecia areata: evaluation of 48 cases after 30 months' follow-up. *Br J Dermatol*. 1996;134:869-871.
17. van der Steen PH, van Baar HM, Perret CM, Happle R. Treatment of alopecia areata with diphenylcyclopropenone. *J Am Acad Dermatol*. 1991;24:253-257.
18. Botham PA, Rattray NJ, Walsh ST, Riley EJ. Control of the immune response to contact sensitizing chemicals by cutaneous antigen-presenting cells. *Br J Dermatol*. 1987;117:1-9.
19. Hull SM, Cunliffe WJ, Norris JF. Alopecia areata treated with diphencyprone: is an allergic response necessary? *Br J Dermatol*. 1990;122:716-717.
20. Vedak P, Kroshinsky D. Squaric acid sensitization is not required for response in the treatment of alopecia areata. *J Am Acad Dermatol*. 2015;73:471-476.
21. Yoshimasu T, Furukawa F. Modified immunotherapy for alopecia areata. *Autoimmun Rev*. 2016;15:664-667.
22. Olsen EA, Hordinsky MK, Price VH, et al. Alopecia areata investigational assessment guidelines—part II. National Alopecia Areata Foundation. *J Am Acad Dermatol*. 2004;51:440-447.
23. Jang YH, Jung HJ, Moon SY, et al. Systematic review and quality analysis of studies on the efficacy of topical diphenylcyclopropenone treatment for alopecia areata. *J Am Acad Dermatol*. 2017;77:170-172.e1.
24. van der Steen PH, van Baar HM, Happle R, Boezeman JB, Perret CM. Prognostic factors in the treatment of alopecia areata with diphenylcyclopropenone. *J Am Acad Dermatol*. 1991;24:227-230.
25. Muthuvel K. Practicality in using diphenyl cyclo propenone for alopecia areata. *Int J Trichology*. 2011;3:96-97.
26. van der Steen PH, Happle R. Topical immunotherapy of alopecia areata. *Dermatol Clin*. 1993;11:619-622.
27. Dall'oglio F, Nasca MR, Musumeci ML, et al. Topical immunomodulator therapy with squaric acid dibutylester (SADBE) is effective treatment for severe alopecia areata (AA): results of an open-label, paired-comparison, clinical trial. *J Dermatolog Treat*. 2005;16:10-14.
28. Buckley DA, Du Vivier AW. The therapeutic use of topical contact sensitizers in benign dermatoses. *Br J Dermatol*. 2001;145:385-405.
29. Case PC, Mitchell AJ, Swanson NA, Vanderveen EE, Ellis CN, Headington JT. Topical therapy of alopecia areata with squaric acid dibutylester. *J Am Acad Dermatol*. 1984;10:447-450.
30. Suarez-Farinas M, Ungar B, Noda S, et al. Alopecia areata profiling shows TH1, TH2, and IL-23 cytokine activation without parallel TH17/TH22 skewing. *J Allergy Clin Immunol*. 2015;136:1277-1287.
31. Lee Won-Soo ASK. Lee Seung-Hun. Comparison in expression of CD1, HLA-DR, and ICAM-1 of follicular keratinocytes from a lesion and a non-lesional scalp of alopecia areata. *Korean J Dermatol*. 1995;66:873-879.
32. Hull SM, Nutbrown M, Pepall L, Thornton MJ, Randall VA, Cunliffe WJ. Immunohistologic and ultrastructural comparison of the dermal papilla and hair follicle bulb from "active" and "normal" areas of alopecia areata. *J Invest Dermatol*. 1991;96:673-681.
33. Cetin ED, Savk E, Uslu M, Eskin M, Karul A. Investigation of the inflammatory mechanisms in alopecia areata. *Am J Dermatopathol*. 2009;31:53-60.
34. Messenger AG, Bleehen SS. Expression of HLA-DR by anagen hair follicles in alopecia areata. *J Invest Dermatol*. 1985;85:569-572.
35. Herbst V, Zoller M, Kissling S, Wenzel E, Stutz N, Freyschmidt-Paul P. Diphenylcyclopropenone treatment of alopecia areata induces apoptosis of perifollicular lymphocytes. *Eur J Dermatol*. 2006;16:537-542.
36. Wasylyszyn T, Kozlowski W, Zabielski SL. Changes in distribution pattern of CD8 lymphocytes in the scalp in alopecia areata during treatment with diphencyprone. *Arch Dermatol Res*. 2007;299:231-237.
37. Avitabile S, Sordi D, Garcovich S, et al. Effective squaric acid dibutylester immunotherapy is associated with a reduction of skin infiltrating T-helper (Th)1 and Th17 cells in alopecia areata patients. *J Dermatol*. 2015;42:98-99.

MATERIALS AND METHODS

Treatment response evaluation

Clinical photographs and a phototrichogram (Folliscope [LeedM Corporation, Seoul, Republic of Korea]) were taken at the initial visit and at 2- to 4-month intervals thereafter, and the involved scalp area was evaluated using the Severity of Alopecia Tool score.[S1] The phototrichogram was used supplementally to observe local changes in lesions, including counts of terminal and vellus hair and hair thickness. Treatment response was divided into 4 categories by calculating percent scalp hair regrowth solely on the basis of extent of absolute hair loss: complete response (CR) (>90% regrowth), partial response (50%-90% regrowth), inadequate response (10%-50% regrowth), and no response (0%-10% regrowth).

Relapse was evaluated in patients who showed a CR and was defined as the recurrence of 25% or more hair loss. Patients who showed a CR were classified into a DPCP-continued group and a DPCP-discontinued group and outcomes were compared.

Adverse effects evaluation and management

Adverse effects (AEs) were divided into 4 categories: no AEs (Pruritus Visual Analog Scale [PVAS] score of 0 and no erythema and scaling), mild AEs (PVAS score of 1-3 with or without mildly increased erythema), moderate AEs (PVAS score ≥4 with or without increased erythema and scaling), and severe AEs (vesicles or lymph node enlargement). Patients without AEs or with mild AEs were not prescribed medication. Patients with moderate-to-severe AEs were prescribed an antihistamine to control symptoms. Among the antihistamines, ebastine and fexofenadine, which have been reported to be associated with hair regrowth in patients with AA, were prescribed.[S2,S3] Medium-potency topical steroids were prescribed for those patients who displayed scaling and eczema after DPCP treatment, with a recommendation for use on a short-term basis to control discomfort.

Immunohistochemical staining

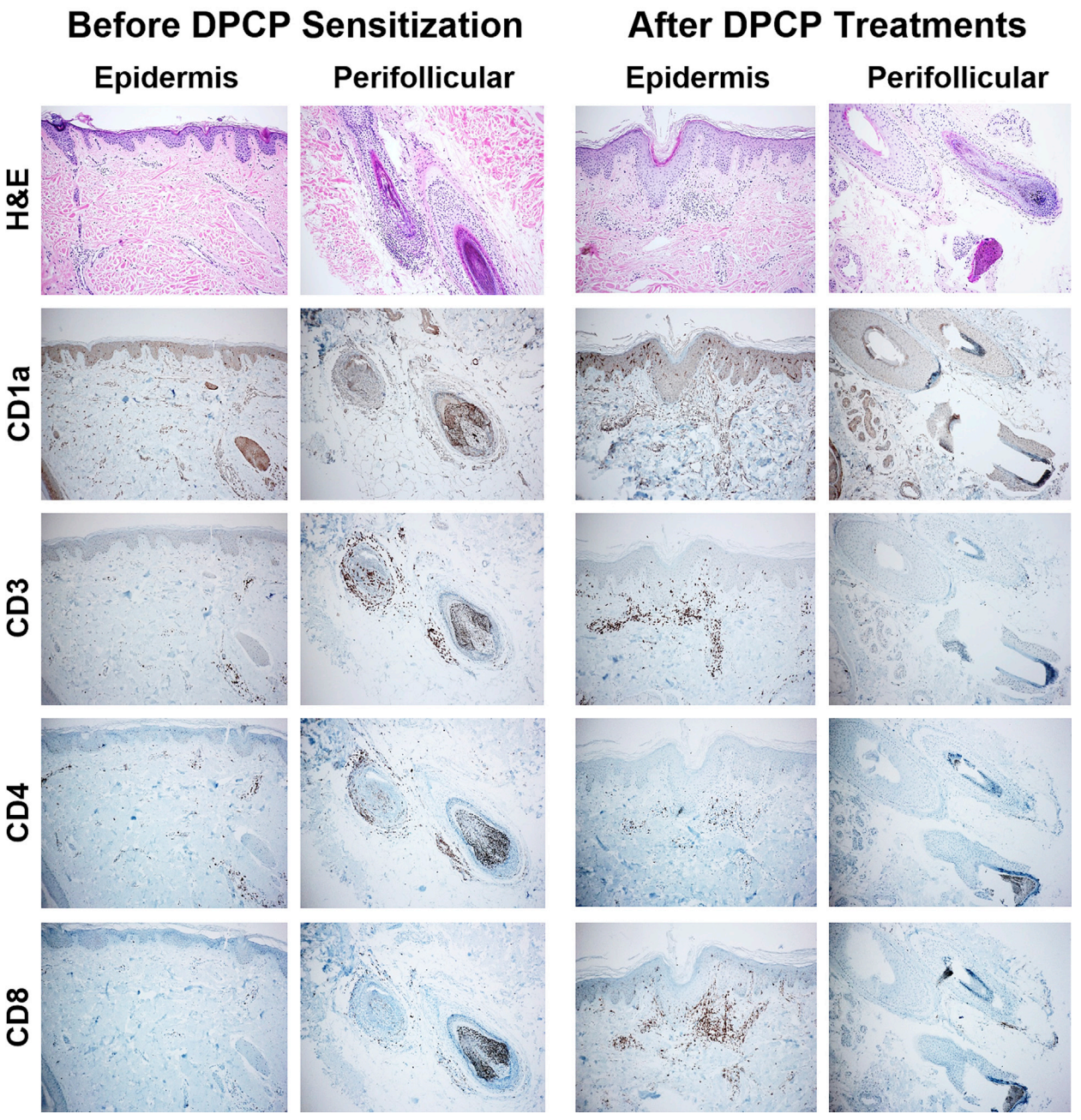
Immunohistochemical staining was performed to assess expression of CD1a, CD3, CD4, and CD8 cells. Briefly, 5-μm-thick paraffin sections were incubated with primary antibodies against CD1a (NeoMarker, CA), CD3 (Dako, Carpinteria, CA), CD4 (Ventana Medical Systems, Tucson, AZ), and CD8 (NeoMarker) overnight at 4°C. After 3 wash cycles, the sections were incubated with anti-rabbit secondary antibody for 30 minutes. Staining was detected with an ABC Peroxidase kit (Vector Lab, Burlingame, CA) while counterstaining with hematoxylin was performed. Representative images for each group are shown.

REFERENCES

S1. Olsen EA, Hordinsky MK, Price VH, et al. Alopecia areata investigational assessment guidelines—part II. National Alopecia Areata Foundation. *J Am Acad Dermatol*. 2004;51:440-447.

S2. Ohyama M, Shimizu A, Tanaka K, Amagai M. Experimental evaluation of ebastine, a second-generation anti-histamine, as a supportive medication for alopecia areata. *J Dermatol Sci*. 2010;58:154-157.

S3. Inui S, Nakajima T, Toda N, Itami S. Fexofenadine hydrochloride enhances the efficacy of contact immunotherapy for extensive alopecia areata: retrospective analysis of 121 cases. *J Dermatol*. 2009;36:323-327.



Supplemental Fig 1. Histologic and immunohistochemical staining before sensitization and after 3 treatments with diphenylcyclopropenone (DCPC). Skin biopsies were performed to investigate whether modified DPCP treatment resulted in a desired immune response. Specimens were taken from the lesions before DPCP sensitization and after treatments with 0.01% DPCP once a week for 3 weeks. Hematoxylin and eosin (H&E) staining was performed and immunohistochemical staining was used to assess the expressions of CD1a, CD3, CD4, and CD8 (n = 3).

Supplemental Table I. Logistic regression analysis of disease- and treatment-related factors associated with treatment response

	Response grade		Unadjusted		Adjusted	
Characteristic	Good (CR + PR)	Poor (IR + NR)	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Alopecia type						
Patchy	85	29	Ref		Ref	
Alopecia totalis	4	3	2.198 (0.464-10.411)	.321	1.019 (0.192-5.395)	.983
Alopecia universalis	16	22	4.030 (1.867-8.701)	<.001	3.137 (1.385-7.107)	.006
Sex						
Male	62	31	1.070 (0.550-2.080)	.842	—	
Female	43	23				
Age at onset, y						
Childhood onset (<13 y)	10	4	1.316 (0.393-4.408)	.656	—	
Adult onset (≥13 years)	95	50				
Age at visit, y						
<40	54	24	1.002 (0.517-1.944)	.995	—	
≥40	51	30				
Disease duration before DPCP treatment, mo						
<22	89	35	3.020 (1.396-6.531)	.005	0.570 (0.236-1.376)	.211
≥22	16	19				
ANA abnormality						
With	8	7	1.806 (0.618-5.278)	.280	—	
Without	97	47				
Autoimmune disease history						
With	4	1	0.690 (0.228-2.091)	.512	—	
Without	101	53				
Atopic dermatitis history						
With	6	2	0.635 (0.124-3.256)	.586	—	
Without	99	52				
Family history						
With	6	1	0.311 (0.037-2.654)	.286	—	
Without	99	53				
Severity at first visit						
Mild (0%-24%)	39	12	1.649 (1.218-2.232)	.001	1.459 (1.033-2.060)	.032
Moderate (25%-49%)	27	8				
Severe (50%-74%)	24	14				
Total (75%-100%)	15	20				
Highest DPCP treatment concentration						
0.01%	39	17	1.414 (0.997-2.006)	.052	1.435 (0.922-1.952)	.124
0.025%	43	18				
0.05%	18	10				
0.1%	5	9				
Adverse effects						
None	43	18	Ref		—	
Pruritus only	47	31	1.387 (0.698-2.756)	.350		
Erythema, eczema, etc	15	5	0.612 (0.210-1.785)	.369		
Medication because of AEs						
No medication	68	33	Ref		—	
po antihistamine only	29	18	1.170 (0.594-2.304)	.651		
po antihistamine + topical steroid	8	3	0.713 (0.181-2.805)	.629		

AE, Adverse event; *ANA*, antinuclear antibody; *CI*, confidence interval; *CR*, complete response; *DPCP*, diphenylcyclopropenone; *IR*, inadequate response; *NR*, no response; *OR*, odds ratio; *po*, by mouth; *PR*, partial response; *Ref*, reference.

Supplemental Table II. Frequency of relapse according to maintenance treatment

	Relapse		
DPCP treatment	**No**	**Yes**	**Total**
DPCP continued, n (%)	21 (45.7)	10 (21.7)	31 (67.4)
DPCP discontinued, n (%)	5 (10.9)	10 (21.7)	15 (32.6)
Total	26 (56.5)	20 (43.5)	46 (100)

Odds ratio, 4.20; 95% confidence interval, 1.13-15.59; $P = .027$.
DPCP, Diphenylcyclopropenone.