# Treatment of Alopecia Areata With Topical Sensitizers

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***Abstract:*** **For those with severe alopecia areata, with greater than 50% scalp involvement, topical immunotherapy with diphenylcyclopropenone or squaric acid dibutylester is considered the treatment of choice. This article not only reviews the safety and efficacy of topical sensitizers for the treatment of alopecia areata but also highlights strides that have been made in the literature concerning their use in pediatric populations, molecular mechanisms of efficacy, and improved safety through targeted delivery methods.**

A multitude of dermatologic conditions involve immune-mediated processes. As such, many treatments in dermatology fall into the category of immunosuppression or immunomodulation. Topical sensitizers, which serve as haptens for allergic contact dermatitis, produce a delayed-type hypersensitivity reaction and have been a useful therapeutic modality in the treatment of many dermatologic conditions, including verruca vulgaris (VV) and alopecia areata (AA).

An ideal therapeutic topical sensitizer has the following characteristics: (*a*) is safe, (*b*) is absent from the natural environment, (*c*) and has little potential to induce cross-sensitization to other substances. The first topical sensitizer described was *tris*-ethyleniminobenzoquinone (trenimon), which was used to treat basal and squamous cell carcinoma.[1] It has since fallen out of favor because of its mutagenic properties.[2] Poison ivy was historically used to treat AA[3] but was not an ideal topical sensitizer because it is commonly found in the United States and has cross-reactivity with poison oak and sumac. Nickel, formalin, and primin have been used in the past for both AA and VV but are also not ideal sensitizers because they are not consistently reliable antigens and are also commonly found in the environment.[4]

The adverse effects are similar among the various topical sensitizers. The most common adverse effects are vesicular/bullous reactions (Fig. 1),[5,6] cervical and occipital lymphadenopathy,[6–8] facial and scalp edema, contact urticaria,[8–10] flulike symptoms, erythema multiforme–like reactions,[7,11] and pigmentation changes, including vitiligo.[8–10]

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## TOPICAL SENSITIZERS

In general, there are two main management options for AA: immunosuppression and immune deviation. Immunosuppression is generally the strategy of choice in patients with acute and rapidly progressive AA, whereas immune deviation, with topical sensitizers, is favored in patients with chronic relapsing AA.[12,13] The two cornerstones of topical sensitization in AA treatment remain squaric acid dibutylester (SADBE) and diphenylcyclopropenone (DPCP). Diphenylcyclopropenone and SADBE have been shown to have comparable efficacy results and response rates after comprehensive review of topical immunotherapy studies.[14] Diphenylcyclopropenone, however, is considered by some to be the most effective form of immunotherapy and is generally considered the first-line treatment for AA totalis.[15,16]

### Diphenylcyclopropenone

Diphenylcyclopropenone is one of the most commonly used and well-studied topical sensitizers. It was first synthesized in 1959 and has since been used in the treatment of multiple immune-mediated dermatologic diseases.[17] There seems to be no cross-sensitivity with other chemicals, except for alpha, alpha(1)-dibromodibenzylketone, which is its precursor.[18] Diphenylcyclopropenone is also nonmutagenic in the Ames assay at the concentrations of 50 and 100 μg/mL.[19] Alpha, alpha(1)-dibromodibenzylketone, however, has been found to be mutagenic in vitro and is a potential contaminant in DPCP.[20] Diphenylcyclopropenone has not been shown to be teratogenic; however, it is currently still recommended that pregnant women avoid contact with it. The University of British Columbia Hair Clinic reported 6 women who became pregnant while being treated with DPCP, and all children were born normal.[16] Recent studies have clearly demonstrated that DPCP has the ability to induce immunomodulatory molecules in healthy skin, most notably CTLA4, IDO, and Foxp3, which have been identified as important factors in the pathogenesis of AA.[21] It is currently the preferred topical sensitizer because it is cheaper and more stable in acetone.[22] Success rates for DPCP reported in the literature are highly variable, ranging anywhere from 4% to 85% in terms of acceptable regrowth.

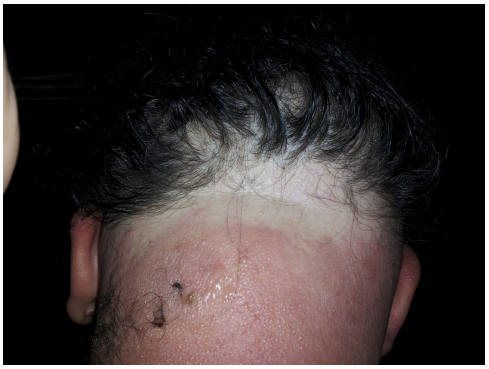

**Figure 1.** Bullous reaction to DPCP application in a patient with ophiasis pattern AA.

### Squaric Acid Dibutylester

Squaric acid dibutylester was first found to be a contact allergen in 1979 and has been used to treat AA, VV, and even skin cancers. Although some literature does support SADBE as an efficacious treatment option for AA either as monotherapy or in combination with other therapies, the strongest evidence for its use lies with SADBE being used as part of a combination therapy regimen.[23–25] Squaric acid dibutylester is also reliably allergenic and environmentally absent. Success rates for SADBE have been shown to lie between 50% and 60% in terms of acceptable cosmetic regrowth.[26]

## MECHANISM OF ACTION OF TOPICAL SENSITIZERS

Topical sensitizers act as haptens to initiate a delayed-type (type IV) hypersensitivity reaction by binding to an endogenous protein to create a complete antigen. This is clinically manifested as allergic contact dermatitis.[27] The hapten-peptide complex is then taken up by either Langerhan cells in the epidermis or dendritic cells in the dermis. The antigen-presenting cell matures as it travels to skin draining lymph nodes and activates an antigen-specific T-cell receptor through antigen recognition.[27] The activated T cell proliferates and expresses new molecules including the cutaneous lymphocyte-associated antigen (CLA). This molecule binds to E-selectin, which is an endothelial ligand that is upregulated in the microvasculature during cutaneous inflammation.[27] Diapedesis then occurs through the expression of various chemokines including CTACK/CCL27, which is a skin-limited chemokine ligand that binds to the chemokine receptor CCR10 found in basal keratinocytes.[28] The effector T cell then causes cytolysis after coming into contact with the hapten-modified peptides on the major histocompatibility complex molecule. This results in damage to the tissue, spongiosis, and inflammation, giving the physical findings of dermatitis.[27]

Diphenylcyclopropenone is well accepted as an immunomodulatory therapeutic and first-line topical sensitizer for disorders including AA, although its molecular effects in the skin are still not fully understood. Multiple theories have been suggested for the mechanism of DPCP in the treatment of AA, all of which recognize the T lymphocyte as the most significant player in the development of hair loss and, in turn, the mechanism of action of DPCP. Although few studies have looked specifically at the immune alterations induced in the scalp that are associated with hair regrowth, currently identified effects of DPCP include decreased ratios of $CD4^+$ to $CD8^+$ T cells[29] and alterations in cytokine profiles of treated scalps. Specifically, Price[30] hypothesized that interleukin-10 secretion from the basal keratinocytes or lesional T cells after application of DPCP results in an inhibitory effect on lesional T lymphocytes.

A recent study from Gulati et al[31] serves as one of the first investigations that has been able to specifically characterize the molecular effects of DPCP in healthy human skin, showing distinct molecular responses at both peak and resolution phases. Their data demonstrated that response to DPCP evolves from an initial inflammatory reaction during peak response at approximately 3 days to a more regulated immune response during the resolution phase of reaction at 14 days, with demonstrated decreased expression of T-cell cytokines, higher levels of transcripts associated with a negative immune response, and the expression of genes exclusively during resolution phases that have been linked to T-regulatory cell development. Results provide support for previous evidence that negative immune regulation plays a significant role in its efficacy. The data also support newly discovered roles for other regulatory molecules, not previously reported, although any potential relevance to treatment of AA was not elucidated.[31]

Overall, consensus lies with local immunomodulation as the most likely mode of therapeutic effect by means of two mechanisms: (1) antigenic competition with the causative antigen, which is still yet to be determined, providing an alternative immune target; and (2) alteration in cytokine profiles after prolonged immune stimulation, which increases T-regulatory lymphocytes to decrease follicular immune reaction.[32] Comparing topical sensitizers with agents that act solely as irritants, such as tretinoin, it becomes clear that the therapeutic effects of DPCP are not caused by the irritating effects of the immunotherapy but, instead, the allergic response. Studies using solely irritants for therapy demonstrate no hair regrowth, whereas studies showing hair regrowth after DPCP have identified allergic reaction as a necessity for regrowth.[33,34]

## USE OF TOPICAL SENSITIZERS FOR TREATMENT OF AA

Alopecia areata is the most common cause of inflammatory hair loss and affects approximately 4.5 million people in the United States.[35] It is often recalcitrant to therapy, and there is no current curative therapy.[36] Alopecia areata presents as nonscarring alopecia in a well-circumscribed area of normal skin, most commonly occurring on the scalp and beard.[37–39] The onset is rapid, and the severity may range from loss of hair in a small single patch to loss of hair on the whole scalp (AA totalis) or even the entire body (AA universalis).[40] Alopecia areata may be associated with other autoimmune conditions, including lupus erythematosus in 0.6% of

patients,[37] vitiligo in 4%,[41] and autoimmune thyroid disease in 8% to 28%.[42] Atopic dermatitis is also associated with AA and is present in 39% of cases.[43]A skin biopsy may be used to aid in the diagnosis of cases where the presentation is atypical.

The pathogenesis of AA is not yet fully understood. It has been hypothesized that it occurs when the constitutive immune privilege of the hair follicle is disrupted.[44,45] The disease process occurs when proinflammatory signals (interferon-gamma and substance P) are present[45–47] in a genetically susceptible individual, leading to the upregulation of major histocompatibility complex class Ia expression in the hair follicle epithelium.[47,48] This ultimately leads to lymphocytic infiltration of the hair follicle, with resultant alteration in the hair cycle. Loss of the hair only occurs during the anagen (growth) phase of hair production. Because of this, it is suspected that the autoantigen responsible is present only during the anagen phase.[45,49–51]

Presently, there is no specific therapy approved by the US Food and Drug Administration for treatment of AA. Because of an unpredictable course consisting of spontaneous remissions and relapses, effectiveness of therapeutics has been historically difficult to study. Hair regrowth cannot always be clearly identified as a sign of treatment response compared with the natural course of the disease. These aspects have made assessment of treatment efficacy difficult.[32] Currently, there exist no randomized controlled trials looking at efficacy of topical immunotherapy for AA, but observational studies have used half-head control methods to distinguish between spontaneous regrowth and response. A comprehensive review of published topical sensitizer studies by Rokshar et al[26] from 1998 demonstrated scarce results (SADBE, 13 trials; DPCP, 17 results), with little being added to this list since (Table 1). To date, therapy of choice for AA is based largely on empirical criteria, taking into account the patient's age, percentage of affected areas, and disease phase (acute vs chronic). Although sparse, evidence-based criteria do give significance to the efficacy of topical immunotherapy with DPCP and SADBE, along with intralesional and topical steroid therapy.[32]

The results from multiple studies in adult patients have provided evidence that DPCP is an effective and well-tolerated treatment for AA. Cotellessa et al[52] examined 56 patients with extensive AA and found that total regrowth of terminal hair was achieved in 48% of patients, with 60% of patients having a persistent response at 6 to 18 months of follow-up. Another trial of 148 patients found acceptable regrowth in 17% of patients suffering from AA totalis or universalis, a 60% regrowth rate for patients with 75% to 99% hair loss, and a 100% regrowth rate for patients with less than 50% hair loss.[49] A lag time of 3 months was common before the development of any significant regrowth, and relapse after significant regrowth occurred in 62% of patients.[49]

In contrast, a study by Ito et al[48] showed that patients with AA who have less than 40% scalp involvement showed no statistically significant response rates when comparing topical immunotherapy with placebo. A similar study showed that DPCP had no improvement from triamcinolone acetonide injections in lesions smaller than 50 $cm^2$ but does provide superior results in areas larger than 50 $cm^2$.[53] This finding led the authors to recommend the use of topical immunotherapy to be restricted to more extensive disease (>40%).[22] There seems to be no synergistic benefit of combining immunotherapy with other traditional therapies including minoxidil,[54] photochemotherapy,[55] and pranobex.[56] There have been 2 case reports, however, of patients with long-standing treatment-refractory AA totalis who developed dense hair regrowth after 1 month of starting a combination therapy of simvastatin 40 mg and ezetimibe 10 mg, along with continued intralesional corticosteroid injections.[57] Although there have not been studies examining the use of the combination of simvastatin and ezetimibe with topical immunotherapy, simvastatin and ezetimibe have been previously described to have a synergistic immunomodulatory effect, which may have contributed to the patients' clinical response.[57]

## Limited Use of Topical Sensitizers in Pediatric Populations

Although DPCP has repeatedly demonstrated both safety and efficacy in the treatment of refractory and advanced AA in adults, limited data are present for children, with current recommendations discouraging use in children younger than 10 years because of the absence of data. Few studies that have examined DPCP for the treatment of severe AA in children have resulted in hair regrowth rates ranging from 27% to 33%.[7] In the largest reported study looking at treatment response to DPCP in 108 children, aged 4 months to 18 years, 13% had complete hair regrowth at 6 months and 25% had partial. Follow-up at 12 months showed 11% with complete regrowth versus 21% with partial. Adverse effects were slightly more common in children younger than 10 years, although side effects warranting discontinuation were overall uncommon.[58]

## Treatment Prognosis Factors

There are five major prognostic factors that have been shown to be significant in the outcome of treatment. These factors are (1) the type of AA (patchy, ophiasis, diffuse, totalis, universalis); (2) age of onset; (3) presence of nail changes; (4) duration of AA before treatment; and (5) the association with atopic dermatitis, with more favorable prognosis correlating with patchy-type AA, older age of onset, the absence of nail changes, shorter duration of disease before treatment onset, and a negative history of atopic dermatitis.[59,60] However, these prognostic factors have not been shown to be associated with a greater likelihood of partial or complete response to DPCP therapy in children.[58] If there is no hair growth after 20 to 30 weeks, the patient is considered to have failed topical immunotherapy.[61,62]

## Treatment Protocol for AA With DPCP

Although there are no data available on different treatment protocols for the use of DPCP for AA, our institution follows the protocol published by Buckley and Du Vivier[63] very closely. A sensitization site is chosen first within the affected area of the scalp.

**TABLE 1. Studies Using DPCP in Immunotherapy for AA (Since 1998)**

| Author(s)/ Year | Control | No. Patients | Age Range/ Mean Age | Mean Duration of Disease | Form of AA | Duration of Therapy | Duration of Follow-up | Response Rate | Complete + Partial Response (CR/PR)* | Relapse Rate |
|---|---|---|---|---|---|---|---|---|---|---|
| Donovan et al[58]/2012 | No | 108 | 4 mo–18 y (11.7 y) | 3.8 y | AA | 6 mo | 12 mo | 13% CR<br>25% PR | 38% | 6% |
| Galardi et al/2003 | No | 21 | ? | ? | AA | 6 mo | None | 71.4% | 71.4% | ? |
| Wiseman et al[49]/2001 | No | 148 | 8–77 y (36.3 y) | 9.6 y | AT/AU-35; 75%–99%<br>AA- 48; 50%–75%<br>AA-39; 25–50%<br>AA- 26 | 7.2 mo | 37 mo | 17%-AT/AU;<br>60%- 75%–90% hair loss;<br>100%- <50% loss++ | ? | 62% |
| Contellessa et al[56]/2001 | Yes | 56 | 18–50 y (23 y) | 1–10 y (mean, 6 y) | AA scalp- 42 >90% scalp; 14- patchy | 6–12 mo | 6–18 mo (mean, 12 mo) | 48% CR | ? | 19% |

**Complete response is defined as full regrowth. Partial response is defined by anything but full regrowth.*
*++Response rate was judged as cosmetically acceptable response being anything greater than 75% regrowth.*

Although sensitizing on the arm is an option as well, there tends to be an increased risk of adverse reactions during treatment.[64,65] A cotton swab is dipped into 2.0% DPCP in acetone and applied to a small patch of alopecia approximately 2 × 2 cm in size. The patient is advised not to wash the site for at least 48 hours and returns to the clinic in 1 to 2 weeks. Any sign of eczematous reaction within the first 2 weeks is an indicator that sensitization was successful. Occasionally, vesiculobullous reactions have been noted (Fig. 1).

Once sensitization has taken place, the ideal dose of DPCP must be determined by controlled titration. The initial concentration used varies from 0.001% to 0.1%, depending on the strength of the sensitization response. A patch of alopecia is painted with the initial dose of DPCP and, as with sensitization, should be kept out of water and protected from light for approximately 48 hours after application. The patient returns weekly to determine the extent of reaction, and the dose is increased if the appropriate reaction is not observed. The ideal dose will induce mild erythema and pruritus as would be expected from a contact dermatitis but should not be strong enough to induce vesicle or bulla formation. We recommend using concentrations of 0.001%, 0.01%, 0.1%, 1.0%, and 2.0% to titrate the correct dose.

After determining the ideal dose, DPCP is applied to one half of the scalp (or selected areas of alopecia) every 1 to 2 weeks for approximately 12 weeks. Depending on the patient, it may be more convenient to send a prescription of the correct dosage to a compounding pharmacy. Treatment should be extended to the entire scalp once the initial areas treated show hair growth.[61] If a lack of response is noted after 20 to 30 weeks, this is considered a treatment failure.[61] If hair growth remains stable for more than 3 months, discontinuation can be begun in a stepwise fashion.[62] Eyebrows can also be treated as above,[64] but it is not recommended to use DPCP on eyelashes because of the proximity to the eyeball.[62]

## DISCUSSION

For those with severe AA, with greater than 50% scalp involvement, topical immunotherapy with DPCP is considered the treatment of choice. Although there is still a lack of randomized controlled trials surrounding the use of DPCP in AA, observational studies show strong evidence for both safety and efficacy of its use in adults. The use of DPCP in children is an area that lacks significant data at this point in time, yet the few studies that do exist demonstrate acceptable efficacy and safety in children aged 4 months to 18 years.[58]

Although molecular mechanisms of DPCP efficacy in the treatment of AA have not been fully elucidated, recent investigations into molecular characteristics of response show significant evidence for the importance of negative immune response mechanisms. New roles of various regulatory molecules have been uncovered, but work still needs to be done to reveal any potential relevance to AA treatment.

Evidence described in the literature has justified the adverse events seen after the use of DPCP, although strides have been made

to increase the safety of its use. Most recently, Lin et al[66] report the successful formulation of nanostructured lipid carriers loaded with squalene, which they were able to show enhanced percutaneous absorption and follicular penetration, with subsequent decreased side effect profiles for patients. Such developments suggest that feasible alternatives for a more controlled DPCP delivery system may be possible moving forward.

Despite the benign nature of AA, it can have serious aesthetic consequences for patients. First episodes often tend to self-resolve within a year; however, relapse is not uncommon, even after several years. Although the wait-and-watch method is currently recognized as an inferior method of treatment because of increasing evidence of effective treatment options, including topical sensitizers, practitioners should use caution when attempting extreme treatment regimens in circumstances of nonresponsive cases.

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