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REVIEW

Application of Topical Immunotherapy in the Treatment of Alopecia Areata: A Review and Update

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Abstract: Treatment of extensive or recalcitrant alopecia areata (AA) is a major clinical challenge. Even after thorough investigation of several medications, its treatment outcomes have remained unsatisfactory. While there is no US Food and Drug Administration-approved medication for AA yet, topical immunotherapy has been a well-documented treatment option. Dinitrochlorobenzene, squaric acid dibutylester, and diphenylcyclopropenone are three substances that have demonstrated efficacy in the treatment of extensive or recalcitrant AA. Despite being commonly used, the mechanism underlying topical immunotherapy is not well-elucidated and a wide range of clinical efficacies have been reported in the literature. The aim of this review was to summarize and update the pharmacology, mechanism of action, therapeutic efficacy, and tolerability of topical immunotherapy in the treatment of AA.

Keywords: contact sensitizers, dinitrochlorobenzene, diphencyprone, diphenylcyclopropenone, hair loss, squaric acid dibutylester

Introduction

Alopecia areata (AA) is a common nonscarring alopecia with a worldwide prevalence of 0.1–0.2%. It is characterized by a patch or multiple patches of hair loss, diffuse or complete hair loss, occurring on the scalp or other body areas and presenting with or without exclamation mark hairs.[1,2] AA is theorized as a hair follicle-specific autoimmune disease with a genetic predisposition, progressing from disruption of immune privilege of hair follicles.[3] Due to its nature, up to 25% of patients are recalcitrant to therapy and recurrence rate is as high as 13.5–33% in patients.[4] Several therapeutic modalities have been examined to date; however, treatment responses were variable and unpredictable.[5–10] Moreover, side effects from medications used, especially from corticosteroids, are major concerns in long-term therapy. Although no treatment for AA has been approved by US Food and Drug Administration yet, appropriate modalities are essential considering the associated psychological consequences, including anxiety and depression.[11,12]

Currently, topical immunotherapy offers the best efficacy and safety regarding long-term treatment of patients with recalcitrant AA.[13] It enables hair regrowth by inducing an inflammatory response to contact sensitizing agents at the applied area. Most of the published studies have reported response rates between 9 and 87%, with an effective rate of approximately 30%, which is considered worthwhile.[14] Regardless of its popularity, the underlying mechanism of AA treatment is not fully

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elucidated. The aim of this review was to summarize and update the pharmacology, mechanism of action, clinical efficacy, and adverse events of topical immunotherapy in the treatment of AA.

Role of Pro-inflammatory Cytokines in Pathogenesis of Alopecia Areata

The pathomechanism of AA involves the complex interaction between innate and adaptive immunities.[15] Its immunohistochemistry showed peri- and intrafollicular infiltration of $CD4^+$ and $CD8^+$ T cells at the bulb of affected the hair follicle. While $CD4^+$ T cells were located mostly in the peri-follicular area, $CD8^+$ T cells penetrated to the intrafollicular area, thereby disrupting the hair growth cycle.[3] Recent hypothesis of the disease mechanism focuses on the collapse of immune privilege of hair follicles and autoreactive lymphocytes. Under normal conditions, hair follicles form an area where autoantigens cannot be recognized due to the lack of major histocompatibility complex (MHC) in the proximal outer root sheath and matrix cells.[16,17] In AA, the immune privilege is disrupted by specific triggers, such as microtrauma, viral infection, or endocrine dysfunction, resulting in immune dysregulation.[18] Further, ectopic expression of MHC class I, recognized by autoreactive $CD8^+$ T cells, could directly and adversely affect anagen hair follicles, leading to follicular apoptosis.[18]

Several studies have demonstrated the role of inflammatory cytokines, especially Th1-mediated cytokines, in the occurrence of AA via two possible mechanisms, either activation of the $CD8^+$ T cell pathway or induction of cessation of hair growth cycle. Interferon (IFN)-γ, the hallmark cytokine of Th1-mediated pathway, is regarded as a key cytokine in AA.[16,19] A large amount of IFN-γ is produced by autoactivated $CD8^+$ cells and antigen presenting cells (APCs) after initial inflammatory insult on hair follicles,[20–27] resulting in further upregulation of MHC class I and II molecules in the bulb of hair follicles and activation of $CD8^+$ and $CD4^+$ T cells.[28] Serum from patients has been reported to contain a higher level of IFN-γ, interleukin (IL)-2, IL-12, and IL-18 compared to that from control subjects.[29,30] Serum levels of IFN-γ tend to be elevated with disease severity.[19,31] IL-1B, IL-2, and IL-6 are also present in human scalp lesions.[32] Furthermore, the Th17 pathway may be involved in disease development by collaboration with the Th1 pathway via IL-17A and IL-17F.[33]

Recent studies have reported significantly increased serum levels of Th2 cytokines, such as IL-4 and IL-10, which were suspected to be critical players in disease suppression. Serum level of IL-4 was found to be higher in patients with patch-type AA, a mild form of the disease, than in those with other subtypes.[29] Apart from the Th2-mediated pathway, regulatory T cells are also responsible for the suppression of exaggerated Th1- and Th2-related inflammation via TGF-β and IL-10.[31] Without a definite conclusion about its mechanism, the majority of the studies have shown no significant difference in regulatory cytokines between patients with AA and normal controls.[31,34–40]

Topical Immunotherapy and Alopecia Areata

Topical immunotherapy has been adopted for the treatment of several immune-mediated skin diseases since the 1970s. This modality is recommended as the first-line treatment in severe and recalcitrant AA due to its high efficacy and relatively low rate of adverse effects, as shown in previous reports.[5,41–44] The potential role of topical immunotherapy in the treatment of AA has been demonstrated for decades and various substances have been introduced for the purpose.[45] In this review, we have emphasized three commonly prescribed agents, namely dinitrochlorobenzene (DNCB), squaric acid dibutylester (SADBE), and diphenylcyclopropenone (diphencyprone, DPCP).[46]

Mechanism of Topical Immunotherapy in Alopecia Areata

Various theories exist regarding the mechanisms of immunotherapy for the treatment of AA. The main mechanism focuses on antigenic competition using immunomodulators to induce allergic contact dermatitis at the applied area through delay-type hypersensitivity.[47] The substance acts as hapten, which binds to endogenous protein, forming a complete antigen. This complex is detected by APCs, and activates antigen-specific T cells causing the clinical condition of dermatitis.[48] With the elicitation of an allergic reaction, suppressor T cells are generated, which infiltrate around hair follicles in the late phase. These newly infiltrating T cells, mainly expressing $CD8^+$ and $CD1a^+$, act against the autonomous $CD4^+$ and $CD8^+$ T cell populations, and disturb APC migration at the affected follicles.[49] A decrease in $CD4^+$ T cells and increase in $CD8^+$ T cells in the treated area results in an alteration of lymphocyte perifollicular pattern, with a decrease in the ratio of $CD4^+$ to $CD8^+$

T cells from 4:1 to 1:1. MHC class I and II expression also declined after treatment with topical immunotherapy.[50] A molecular study demonstrated that topical immunotherapy can induce the expression of immunoregulatory molecules, such as cytotoxic T-lymphocyte-associated protein 4, forkhead box P3, and indoleamine 2,3-dioxygenase.[51] Hence, improvement of local immunoregulation could promote hair regrowth in patients with AA.[47] However, all of these T-cell-mediated mechanisms are supposed to be modulated by counteracting inflammatory cytokines and growth factors, especially Th1 cytokines.[16,52]

Another mechanism regarding cytokine alteration has also been proposed. Previous studies had suggested that Th1 cytokines, such as IFN-γ, IL-1β, and IL-2, were elevated in untreated patients with AA. However, after receiving treatment with topical immunotherapy, levels of IFN-γ and IL-12, and Th17 cytokines reduced along with increase in regulatory cytokines, namely IL-2, IL-4, IL-8, and IL-10, and tumor necrosis factor (TNF)-α, in both serum and scalp biopsy specimens.[50,52–54] Application of DPCP was demonstrated to increase mRNA expression of IL-2, IL-8, IL-10, and TNF-α.[34] IL-10 has been theorized as a key factor for the effectiveness of immunotherapy by inhibiting T lymphocytes.[34] However, additional studies would be required to confirm the role of regulatory cytokines in AA, since Lee et al had reported no significant difference in the IL-10 levels of scale samples from patients (responders vs nonresponders) after receiving topical immunotherapy.[55] Gong et al demonstrated that patients with pretreatment showed elevated Th1-cytokines, using IL-12 as the marker, and could be good candidates for DPCP therapy. Patients with elevated Th2-cytokines, such as IL-4, can counteract the immune response elicited by topical immunotherapy, leading to poor treatment response.[56]

In summary, there are two possible mechanisms of topical immunotherapy in the treatment of AA. The first involves antigenic competition, which shifts the target of T cells from hair follicles to the epidermis. The second involves cytokine alteration, which increases in T-regulatory lymphocytes causing decline of follicular immune reaction. However, these two mechanisms would require further evidence in order to establish their roles in the treatment of AA.

Treatment Protocol for Alopecia Areata with Topical Immunotherapy

There is a standard protocol for all topical sensitizing agents used in the treatment of AA. Substances are commercially available either in powder form or as an acetone solution. To preserve their therapeutic properties, the agents need to be stored in amber glass at a temperature of 4°C and kept away from sunlight.[57] Patients are initially sensitized with 2% solution applied on a 4×4 cm area on the upper arm or on the scalp. After two weeks of sensitization, treatment begins with a weekly application of the substance, starting from the lowest concentration (usually 0.01%) on one side of the scalp while the other side serves as the control. It is necessary to avoid sunlight and washing of the scalp for 48 h after the application. Weekly increase in the concentration of substance may be necessary for maintaining optimal eczematous reaction (tolerable itching and erythema). The concentration should be carefully adjusted by following the reaction. Once hair regrowth becomes obvious on the treated side, the substance may be applied on the entire scalp. Hair regrowth is expected between three and six months of treatment and should be discontinued if no regrowth occurs after six months.[58,59]

Dinitrochlorobenzene

DNCB or 1-chloro-2,4-dinitrobenzene was the earliest contact allergen introduced in 1912. Its chemical structure is $C_6H_3Cl\ (NO_2)_2$ (Figure 1). It used to be popular for the treatment of extensive or recalcitrant AA, with hair regrowth ranging around 25–89% and complete regrowth rates being 6.7–25%. However, in 1985, DNCB was found to be mutagenic and carcinogenic in Ames test and had to be discontinued thereafter.[60] Clinical trials using DNCB for the treatment of AA are listed in Table 1.[61–65]

Squaric Acid Dibutylester

SADBE or 3,4-dibutoxycyclobut-3-ene-1,2-dione is a sensitizing agent with chemical structure $C_{12}H_{18}O_4$ (Figure 2). Its efficacy in AA treatment was first reported in

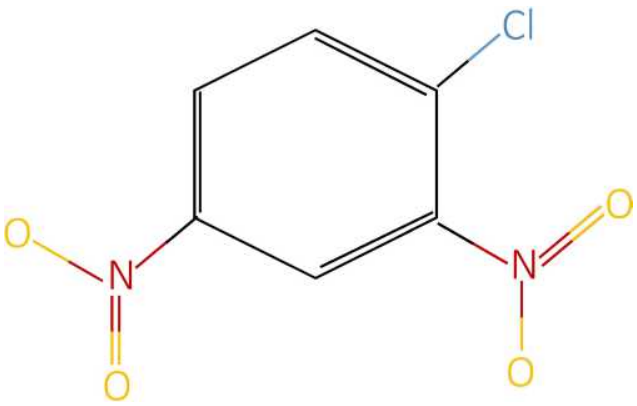

Figure 1 Chemical structure of dinitrochlorobenzene.

Table 1 Characteristics of Clinical Studies Using Dinitrochlorobenzene for the Treatment of Alopecia Areata

Author(s), Year	Study Design	Number of Patients	Age, Years, Range	Type of AA, n	Duration of Therapy, Months	Complete Response, n (%)	Partial Response, n (%)	Relapse Rate, n (%)
Breuillard and Szapiro, 1978[61]	Prospective study	30	6–59	AT=27, other=3	NS	11 (36.6)	10 (33.3)	NS
Friedman, 1981[62]	Prospective study	51	12–72	AT=38	5–6.6	3 (5.8)	27 (52.9)	NS
Swanson et al, 1981[63]	RCT	22 (DNCB=12)	16–68	AT/AU=13, other=9	6	0	7 (63.3) (spontaneous hair regrowth=1)	0
Singla et al, 1991[64]	Prospective study	50 (DNCB=25)	NS	NS	4	4 (16)	12 (48)	NS
Yoshizawa et al, 2002[65]	Prospective study	20	12–64 (mean=31.9)	AT=2, <50%, AA=18	5	5 (25)	10 (50)	NS

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis; DNCB, dinitrochlorobenzene; NS, not stated; RCT, randomized controlled trial.

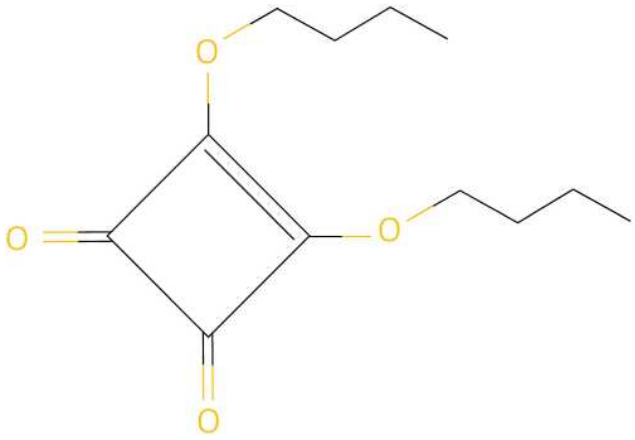

Figure 2 Chemical structure of squaric acid dibutyl ester.

1980.[66] SADBE is an ideal sensitizing agent due to its absence in the environment and lack of cross-reaction with other agents. However, it is unstable in acetone solution and can degrade within a few hours at room temperature. To circumvent this issue, a light-resistant temperature-controlled container is used for sustaining its maximum effect.[67]

The response rate of SADBE in the treatment of AA ranges between 19% and 79.6%. A meta-analysis by Lee et al, including 45 studies with 2227 patients, showed 51.8% of patients with patch-type AA and 25% of patients with alopecia totalis (AT)/alopecia universalis (AU) to have achieved satisfactory hair regrowth.[68] A network meta-analysis by Gupta et al suggested that 35% and 49.7% of patients with patch-type AA and AT/AU, respectively, demonstrated satisfactory hair regrowth.[69]

One randomized placebo-controlled trial of SADBE was published in 1986, which demonstrated complete hair regrowth in 64% of patients with patch-type AA.[70] The largest prospective study of SADBE, conducted in 144 patients with variable severities of AA, reported 80% and 49% response rates in mild and severe forms of AA, respectively.[71] Satisfactory results were reported by Happle et al, Dall'oglio et al, and Chua et al, with complete hair regrowth rates of 87%, 79.6%, and 68%, respectively.[66,72,73] In contrast, Caserio and Gianetti and Orecchia reported unsatisfactory results with complete response rate of 28.5% and 19%, respectively.[74,75] Characteristics of clinical studies using SADBE for the treatment of AA have been summarized in Table 2.[66,68,69,71–82]

Adverse effects of SADBE include redness, swelling, and itching at the application site. However, some patients might experience more severe reactions, such as blistering, burning of the skin, and spreading of rash to other areas.

Table 2 Characteristics of Clinical Studies Using Squaric Acid Dibutylester for the Treatment of Alopecia Areata

Author(s), Year	Study Design	Number of Patients	Age, Years, Range	Type of AA, n	Duration of Therapy, Months	Complete Response, n (%)	Partial Response, n (%)	Relapse Rate, n (%)
Happle et al, 1980[66]	Prospective study	53	6–57	AT/AU=27, other=26	6–15	46 (87)	NS	9 (19.6)
Giannetti and Orecchia, 1983[75]	Prospective study	26	6–32	AT/AU=6, >50% AA=12, <50% AA=8	NS	5 (19)	12 (46)	NS
Tosti et al, 1986[76]	RCT	44	5–74	PA=44	NS	28 (64)	NS	1 (3.5)
Johansson et al, 1986[77]	Prospective study	19	14–55	AT/AU=13, other=6	4	8 (42.1)	11 (58)	NS
Caserio et al, 1987[74]	Prospective study	14	7–66 (mean=16.5)	AT/AU=12, PA=2	6.5–10.5	4 (28.5)	7 (50)	1 (9.1)
Orecchia et al, 1994[79]	Prospective study	28	5–13	AT/AU=11, PA=17	5–10	9 (32.1)	6 (21.4)	14 (93.3)
Tosti et al, 1996[80]	Prospective study	33	6–14	AT=10, AU=23	4–22 (mean=12.8)	10 (30.3)	6 (18)	7 (43.8)
Chua et al, 1996[73]	Prospective study	19	14–42	AT/AU=7, mixed=12	6	13 (68)	2 (11)	33% in AT 11% in AA
Micali et al, 1996[71]	Prospective study	144	5–50 (mean=23)	AT/AU=15, <50%, AA=71, other=58	12	NS	80% less severe form 49% severe form	NS
Iijima and Otsuka, 1997[81]	Prospective study	48	6–57	AT/AU=14, PA=31, AO=3	6	23 (48)	20 (41.6)	NS
Dall'oglio et al, 2005[72]	Prospective study	54	4–71	AT/AU=13, PA=89, AO=5	24–96	43 (79.6) total 73% in adults 100% in children	NS	44%
Lee et al, 2018[68]	Meta-analysis		45 studies (DPCP 31 studies, SADBE 15 studies, DPCP+SADBE 1 study)		Satisfactory hair regrowth (>75% hair regrowth) 38.4% (PA 51.8%, AT/AU 25%) CR 38.4% (PA 50.5%, AT/AU 35%)			
Sakai et al, 2019[82]	Retrospective cohort study	49	3–74	AT/AU=16, >50% AA=20, <50% AA=13	6–12	11 (57.8)	22 (45)	NS
Gupta et al, 2019[69]	Meta-analysis		38 studies (PA 11 studies, AT/AU 27 studies)		35% Satisfactory hair regrowth (>70% hair regrowth) in PA 49.7% Satisfactory hair regrowth (>70% hair regrowth) in AT/AU			

Abbreviations: AA, alopecia areata; AO, alopecia ophiasis; AT, alopecia totalis; AU, alopecia universalis; CR, complete regrowth; DPCP, diphenylcyclopropenone; NS, not stated; PA, patch-type alopecia areata.

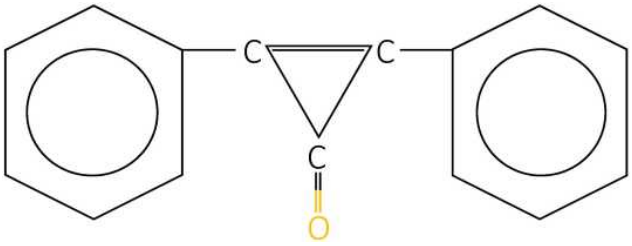

Figure 3 Chemical structure of diphenylcyclopropenone.

Uncommon adverse effects reported include spread of generalized eczema, persistent contact dermatitis, and severe angioedema.[83–86] Postinflammatory hypopigmentation and depigmentation have also been reported after the application of SADBE, and lesions were obviously visible in patients with skin phototype V or VI.[87,88]

Diphenylcyclopropenone

DPCP is a topical sensitizer, efficacy of which was primarily reported by Happle et al in 1983.[89] Its chemical structure is $C_{15}H_{10}O$ (Figure 3). Currently, DPCP is the most commonly used substance owing to the following reasons. First, it is nonmutagenic in Ames assay, with no report of systemic absorption.[45] Second, no long-term adverse effect has been documented yet. Finally, it is less expensive and more stable in acetone solution compared to SADBE.[90] In 2012, the British Association of Dermatologists' guideline also recommended the use of DPCP as the first-line topical sensitizer for the treatment of AA.[42]

Several studies have evaluated the efficacy of DPCP in patients with AA; hair regrowth rate was found to be 6–77%. A systematic review had previously reported an overall hair regrowth rate of 53.75% in DPCP-treated patients.[91] Severity of AA was found to be a significant factor associated with hair regrowth. The highest efficacy of DPCP was reported by Tosti et al with 77% complete hair regrowth in patients with mild AA.[70] The largest retrospective study involving 757 patients with all subtypes of AA was published in 2020.[92] The overall hair regrowth rate has been reported to be 60.1% and the satisfactory hair regrowth (>75% hair regrowth) rate was 16.3%. Comparison across subtypes of AA showed patch-type AA to have 2.56 times higher satisfactory hair regrowth while AU had 2.6 times lower response in comparison to other subtypes.[92] The satisfactory hair regrowth rate of DPCP for patch-type AA was reported ranging between 55.4% and 63.4%.[68,69] When the efficacy of DPCP on AT/AU subtype was considered, two meta-analyses showed different results. Lee et al reported 28.3% of patients with satisfactory hair regrowth while Gupta et al reported a higher rate of 87.9%.[68,69]

Very few studies to date have demonstrated the efficacy of DPCP in children with AA. The efficacy of satisfactory hair regrowth has been reported to range from 11–33%. A prospective study using DPCP in 12 pediatric patients with extensive AA reported initial hair regrowth in 67% of patients and complete hair regrowth in 33% after a mean treatment duration of 7.3 months.[93] Another retrospective study investigated the efficacy of DPCP in 108 children with AA, and found only 13% and 11% of patients to have achieved complete hair regrowth after six and 12 months of treatment, respectively.[94]

Although efficacy of DPCP has been widely investigated for hair regrowth, few studies have focused on the relapse rate after cessation of treatment. A comparative study in this regard showed that patients who continued using DPCP as a maintenance therapy had a lower relapse rate (24.4%) compared to those who did not (68.2%).[95] Hull and Cunliffe reported 63% relapse rate after six months of successful therapy without maintenance treatment. Male gender, high severity of disease, and body hair involvement were established as negative factors determining recurrence.[96] In contrast, a study on 25 patients with complete hair regrowth showed no relapse after discontinuing DPCP, over a mean period of 15 months.[97] The importance of maintenance therapy in topical immunotherapy still remains inconclusive. Clinical studies of DPCP in the treatment of AA have been summarized in Table 3.[43,53,68,69,76,89,91–94,96,98–114]

Diphenylcyclopropenone in Combination with Other Treatments

Use of DPCP as a combination therapy was attempted in several studies for the purpose of enhanced efficacy. Combination therapy using DPCP and anthralin demonstrated higher efficacy over DPCP monotherapy, since one study reported complete hair regrowth in 72% and 36.4% of patients with combined therapy and monotherapy, respectively.[115] On the contrary, two studies and one case series showed nonsuperior efficacy of DPCP and anthralin combination therapy compared to DPCP monotherapy.[116–118] Regarding its combination with 5% minoxidil, Shapiro et al found no significant difference in satisfactory hair regrowth between combined regimen and DPCP monotherapy.[119] When combined with

Table 3 Characteristics of Clinical Studies Using Diphenylcyclopropenone for the Treatment of Alopecia Areata

Author(s), Year	Study Design	Number of Patients	Age, Years, Range	Type of AA, n	Duration of Therapy, Months	Complete Response, n (%)	Partial Response, n (%)	Relapse Rate, n (%)
Happle et al, 1983[89]	Prospective study	27	15–57	AT=22, <50% AA=5	4–17	18 (66.7)	NS	NS
Tosti et al, 1986[76]	Prospective study	35	NS	<40% AA=35	NS	27 (77)	NS	NS
Hull and Norris, 1988[98]	Prospective study	28	18–56	NS	8	8 (28.5)	2 (7.1)	NS
Hatzis et al, 1988[99]	Prospective study	45	9–65 (mean=25.4)	AT/AU=22, other=23	5–8	11 (24.4)	6 (13.3)	6 (35.3)
Hull and Cunliffe, 1989[96]	Observational study	19	11–54	AT/AU=8, >50% AA=8, <50% AA=3	4–17	14 (73.6)	5 (26.3)	12 (63.2)
Monk, 1989[100]	Prospective study	18	10–46 (mean=26.9)	AT/AU=14, >50% AA=4	2–5	6 (33.3)	NS	NS
Ashworth et al, 1989[101]	Prospective study	17	5–72	AT=8, AU=9	At least 7.5	1 (6)	1 (6)	NS
Hull and Cunliffe, 1991[102]	Prospective study	78	NS	AT/AU=45, other=33	At least 8	25 (32)	24 (30)	NS
van der Steen et al, 1991[103]	Prospective study	139	2–69	AT/AU=61, >40% AA=78	NS	70 (50.4)	107 (77)	NS
Hull et al, 1991[93]	Prospective study	12	5–15 (mean=11)	AT/AU=8, other=4	5–12 (mean=7.3)	4 (33.3)	3 (25)	5 (71.4)
Hoting and Boehm,1992[104]	Prospective study	45	14–57 (mean=32)	AT/AU=34, AO=7, >75% AA=4	2.5–44	9(20)	14 (31)	9 (39.1)
Gordon et al, 1996[105]	Prospective study	48	5–64	>90% AA=36, >40% AA=10	18–36 (mean=30.8)	18 (37.5)	9 (18.8)	NS
Schuttelaar et al, 1996[106]	Prospective study	25	4–15 (mean=10.7)	AT/AU=16, <50% AA=10	8–12 (mean=11)	8 (32)	3 (12)	2 (18.2)
Pericin and Trueb, 1998[107]	Retrospective study	68	13–66 (mean=32)	AU=28, other=40	5–64 (mean=15.8)	21 (30.9)	27 (39.7)	13 (27.1)
Cotellessa et al, 2001[108]	Prospective study	52	18–50 (mean=23)	AT/AU=42, PA=14	6–12	25 (48)	11 (21)	10 (27.8)
Wiseman et al, 2001[43]	Retrospective study	148	8–77 (mean=36.3)	AT/AU=35, Other=113	12.2	6 months, 22.5% 1 year, 52%	NS	NS
Aghaei, 2005[109]	Prospective study	27	10–35 (mean=25)	AT/AU=16, >40% AA=11	24	6 (22.2)	16 (59.3)	13 (59.1)
Avgerinou et al, 2008[110]	Prospective study	54	27.2	>25% AA=54	3–24 (mean=6)	20 (37)	15 (27.8)	31 (88.6)

(*Continued*)

Table 3 (Continued).

Author(s), Year	Study Design	Number of Patients	Age, Years, Range	Type of AA, n	Duration of Therapy, Months	Complete Response, n (%)	Partial Response, n (%)	Relapse Rate, n (%)
Ohlmeier et al, 2012[111]	Retrospective study	135	9–75 (mean=36)	AU=13, AT=11, other=111	2–300 (mean=20)	51 (37.8)	20 (14.8)	23 (32.4)
Salsberg and Donovan, 2012[94]	Retrospective study	108	0.33–18 (mean=11.7)	NS	6 and 12	6 months, 14 (13) 1 year, 12 (11)	6 months, 27 (25) 1 year, 3 (21)	NS
Pan et al, 2015[112]	Prospective study	52	NS	AT/AU=15, other=37	10.92	21 (40.4)	6 (11.5)	57.1% with maintenance therapy 85.7% without maintenance therapy
Chiang et al, 2015[113]	Retrospective study	50	3–59 (mean=21)	AT=14, AU=25, other=10	6–180 (mean=36)	19 (39)	13 (27)	17 (34)
Lamb et al, 2016[114]	Retrospective study	133	13–69 (mean=35)	AT=33, AU=20, other=80	NS	21 (15.8)	30 (22.5)	NS
Jang et al, 2017[91]	Systematic review		26 studies		53.7% overall response rate			
					47.6% response rate in AT and AU			
Lee et al, 2018[68]	Meta-analysis		45 studies (DPCP 31 studies, SADBE 15 studies, DPCP+SADBE 1 study)		Satisfactory hair regrowth (>75% hair regrowth) 38.9% (PA 55.4%, AT/AU 28.3%)			
					CR 30.7% (PA 41.6%, AT/AU 22%)			
Gupta et al, 2019[69]	Meta-analysis		38 studies (PA 11 studies, AT/AU 27 studies)		63.4% satisfactory hair regrowth (>70% hair regrowth) in PA			
					87.9% satisfactory hair regrowth (>70% hair regrowth) in AT/AU			
Gong et al, 2020[56]	Prospective study	57	5–63 (mean=27.1)	AT/AU=46, other=38	6	18 (31.5)	NS	13
Manimaran et al, 2020[53]	Prospective study	37	6–51 (mean=27.75)	AT=5, AU=8, other=20	6	9 (27.2)	11 (33.3)	NS
Nasimi et al, 2020[92]	Retrospective study	757	3–77 (mean=25.9)	AT=175, AU=167, other=415	12.9	74 (16.3)	70 (15.4)	NS

Abbreviations: AA, alopecia areata; AO, alopecia ophiasis; AT, alopecia totalis; AU, alopecia universalis; CR, complete regrowth; DPCP, diphenylcyclopropenone; NS, not stated; PA, patch-type alopecia areata; SADBE, squaric acid dibutyl ester.

imiquimod, however, superior efficacy of combination therapy to DPCP monotherapy was evident.[120] Clinical trials using DPCP as a combination therapy for AA are listed in Table 4.[115–120]

Modified Protocol of Diphenylcyclopropenone

Since the standard treatment protocol of contact sensitizers may take a long time until acceptable treatment response is achieved, modified protocols have been introduced to address this issue. Sriphojanart et al and Thuangtong et al had reported the use of a multiconcentration DPCP treatment on patients' scalps at the first visit.[121,122] A concentration that created an optimal eczematous reaction would then be selected as the starting solution for application. Multi-concentration protocol showed similar efficacy as the standard protocol, with a shorter duration till significant hair regrowth is

Table 4 Characteristics of Clinical Studies Using Combination Therapy and Modified Protocol of Diphenylcyclopropenone for the Treatment of Alopecia Areata

Author(s), Year	Study Design	Type of AA, n	Treatment	Results
Combination therapy of DPCP				
Shapiro et al, 1993[119]	RCT	>50% AA=15	DPCP with placebo vs DPCP with 5% minoxidil	DPCP alone: 42.8% response rate DPCP with 5% minoxidil: 33.33% response rate
Durdu et al, 2015[115]	Retrospective study	>50% AA=47	DPCP alone 22 patients vs DPCP with 0.5% anthralin 25 patients	DPCP alone: 36.4% CR DPCP with 0.5% anthralin: 72% CR
Wasylyszyn and Borowska, 2017[120]	Prospective study	Nonresponders=20	DPCP alone 10 patients vs DPCP with 5% topical imiquimod 10 patients	DPCP alone: 40% PR DPCP with 5% topical imiquimod: 50% CR and 40% PR
Nasimi et al, 2019[118]	Retrospective case series	Nonresponders=32	DPCP and 0.5% anthralin	40.62% response rate 27.27% achieved >50% hair regrowth
Ibrahim et al, 2019[116]	Prospective study	>50% AA=24	DPCP alone 12 patients vs DPCP with anthralin 12 patients	DPCP alone: CR 62.5% DPCP with anthralin: CR 18.2%
Kagami et al, 2020[117]	Case series	Refractory AA=4	2% DPCP or SADBE with 0.5% anthralin	25% CR 25% PR 50% no response
Modified protocol of DPCP				
Sriphojanart et al, 2017[121]	Retrospective study	39	Standard regimen 23 patients vs new regimen 16 patients	Standard regimen: 52% response rate New treatment regimen: 50% response rate
Thuangtong et al, 2017[122]	Prospective study	AT/AU=20	Standard regimen vs multilevel DPCP concentration	25% CR within 34 weeks
Lee and Lee, 2018[124]	Retrospective study	80	Home-based treatment: 40 patients vs clinic-based treatment: 40 patients	Home-based treatment: 45% response rate Clinic-based treatment: 45% response rate
Nowicka et al, 2018[123]	Prospective study	39 (AT=9)	DPCP 1-week interval 16 patients vs DPCP 3-week interval 23 patients	DPCP 1-week interval: 46% mean response rate DPCP 3-week interval: 54% mean response rate
Kim et al, 2020[125]	Retrospective study	204	Home-based treatment 51 patients vs clinic-based treatment 153 patients	Significantly lower loss-to-follow-up rate in home-based treatment

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis; RCT, randomized controlled trial; CR, complete regrowth; DPCP, diphenylcyclopropenone; SADBE, squaric acid dibutyl ester; PR, partial response.

achieved.[121] Another study had also investigated the efficacy across various application intervals. Results showed the three-week treated group to have higher efficacy than the one-week interval group, with 54% and 46% response rates, respectively.[123] A subsequent retrospective study reported no significant difference of efficacy between home-based DPCP therapy and clinic-based therapy; however, the home-based group had better compliance compared to the clinic-based group.[124] Clinical trials using modified DPCP therapy for the treatment of AA have been listed in Table 4.[121–125]

Although DPCP is the best-documented treatment for extensive or recalcitrant AA, not all patients achieve a good response, and some might withdraw due to adverse effects. Patients with high serum IgE levels may have more severe adverse events following DPCP application.[56] Regarding safety, most patients were found to be tolerant to DPCP and no systemic absorption has yet been reported.[105] Most adverse effects have been recorded without long-term complications; common side effects include dermatitis and urticaria.[89,98,103–105,108,111,126] Angioedema, anaphylaxis, fever, erythema multiforme-like reactions, postinflammatory hypopigmentation, and depigmentation have been reported as infrequent complications.[99,100,127–129]

Conclusion

Topical immunotherapy is currently considered as the first-line treatment, representing the most effective modality for treating extensive or recalcitrant AA. DPCP and SADBE are currently prescribed as sensitizing agents with appreciable tolerability. Despite being commonly used, the exact mechanism underlying topical immunotherapy for the treatment of AA has not yet been elucidated. Accumulating evidence has shown advantage of topical immunotherapy over no treatment; however, comparison of the efficacies across different clinical studies and different substances is difficult owing to variations of treatment protocols, evaluation methods, and study durations. In our opinion, topical immunotherapy, when used with caution, is an effective and safe treatment. Further studies would be essential for the better understanding of pathophysiology of AA, roles of cytokines for predicting disease and treatment outcomes, and precise pathways via which sensitizing agents function, eventually helping in the establishment of a more effective treatment for individuals with AA.

Disclosure

The authors report no conflicts of interest in this work.

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