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Original Article

Effect of topical immunotherapy with squaric acid dibutylester for alopecia areata in Japanese patients

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AA, alopecia areata; AD, atopic dermatitis;
AT, alopecia totalis; AU, alopecia universalis;
CR, complete response; PR, partial response;
SADBE, squaric acid dibutylester

ABSTRACT

Background: The Japanese guidelines for the treatment of alopecia areata list topical immunotherapies as a drug therapy for this condition. However, there is insufficient evidence of its efficacy to support this recommendation. Thus, we sought to clarify the effect of topical immunotherapy on the progression and severity of alopecia areata in Japanese patients.

Methods: To evaluate the effect of topical immunotherapy with squaric acid dibutylester (SADBE) in alopecia areata patients, we performed a retrospective cohort study on 49 alopecia patients who had received topical immunotherapy with SADBE. Patients were evaluated by the change in alopecia severity at 6 and 12 months after the initiation of topical immunotherapy. The improvement rate was calculated by determination of the complete and partial responses rate to treatment with topical immunotherapy by application of SADBE.

Results: The improvement rate in all alopecia patients treated with SADBE topical immunotherapy was 57.8% (complete response; 11.1% and partial response; 46.7%).

Conclusions: Topical immunotherapy with SADBE is an effective treatment for alopecia areata. Therefore, the current treatment recommendations for alopecia areata with topical immunotherapies are appropriate.

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Introduction

Alopecia areata (AA) appears as various forms of hair loss; in some cases, symptoms can persist due to repeated improvement and recurrence of symptoms and resistance to treatment. In Japan, the “Guideline for the management of AA from Japanese Dermatological Association 2017” was published in 2017.¹ This guideline lists local steroid injection and topical immunotherapy as the first-line therapies for AA. According to this guideline, topical immunotherapy should be the first-line treatment, regardless of age, for AA multilobularis (in which stabilized bald patches affecting 25% or more of the hair-covered scalp), alopecia areata totalis (AT), and alopecia areata universalis (AU). Since we often accept referrals of patients with severe AA, we have great experience of topical

immunotherapy treatment of AA with squaric acid dibutylester (SADBE) for many years. In this study, we have evaluated the effect of topical immunotherapy with SADBE on Japanese AA patients.

Methods

Patients

Based on the medical records of AA patients admitted to our center, we performed a retrospective cohort study. Among patients who visited the Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, at Kumamoto University from 2004 to 2012, 49 patients (21 male, 28 female) who presented with the clinical symptoms of AA received topical immunotherapy with SADBE and could be followed up for 6 months or longer, these patients were included in our study. The patients with alopecia areata monolobularis were excluded because they have a narrow hair loss area and therefore, they did not have any indication for the use of topical immunotherapy.

Sixteen patients were children aged 15 years or younger and the other 33 were aged 16 years or older. The average duration of AA at time of the first consultation was 71 months (2–324 months). The

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mean ages of the onset of AA were 26.0 (range from 3 to 64) years in males and 19.7 (3–74) years in females. The area of hair loss at the time of the first visit was assessed and classified according to the National Alopecia Areata Foundation guidelines.² Hair loss was classified as S₀: no hair loss; S₁: <25% hair loss; S₂: 25%–49% hair loss; S₃: 50%–74% hair loss; S₄: 75%–99% hair loss; S₅: total scalp hair loss, alopecia totalis (AT); and S₅B₂: total scalp and body hair loss, alopecia universalis (AU). Severe AA was defined as S₃, S₄, S₅, or S₅B₂. At the initial visit, 5 patients had S₁ disease, 8 had S₂ disease, 13 had S₃ disease, 7 had S₄ disease, 2 had S₅ disease, and 14 had S₅B₂ disease. At the first visit, each patient was checked to determine whether he or she had a systemic illness which could cause alopecia such as collagen diseases, thyroid diseases, or zinc deficiency. At the beginning of topical immunotherapy treatment with SADBE, written informed consent was obtained.

Methods

Sensitization was performed by applying 1% SADBE to the bald area for 24–48 h. Two weeks later, after confirming that the sensitization, a patch test was performed with 0.1%, 0.01%, 0.001%, 0.0001%, and 0.00001% SADBE. Forty-eight hours after the patch test, minimal erythema concentration was determined and one-tenth of the concentration of SADBE was applied for the first time. Patients were told to visit the outpatient clinic again to confirm whether serious adverse effects such as itching or contact dermatitis developed on the applied area. If mild itching persisted for 2–3 days, it was considered as the appropriate concentration, and this concentration was applied once every 1 or 2 weeks. If persistent itching for 3 days or longer or a severe eczematous reaction was noted, the concentration of SADBE was decreased and the appropriate concentration was re-evaluated 1–2 weeks later. 6 months after the start of topical immunotherapy, the area of hair-loss was evaluated. Regarding the patients who could be followed up, their hair-loss areas were evaluated 1 year later. The disappearance of bald patches was defined as a complete response (CR) and the hair regrowth in 50% or more of the hair loss area was defined as a partial response (PR).² Complete and partial responses were added to calculate the total response. The cases in which 50% or more of the whole scalp was affected (S₃, S₄, AT, AU) were classified as the severe form of AA. Their total response rate and the rate that the area of hair loss decreased to 50% or less of the scalp were calculated. The χ^2 test was used in data analysis to determine the statistical significance of data. The concomitant disorders of the AA patients such as atopic dermatitis (AD), allergic rhinitis, asthma, urticaria, autoimmune disease, thyroid disease, psoriasis, and vitiligo are described in Table 1.

Results

6 months after the first application of SADBE in 5 patients with S₁ disease, 2 were free of disease and 3 maintained S₁ disease.

Among 8 patients with S₂ disease, 1 was free of disease, 2 maintained S₂ disease, and 1 worsened. Among 13 patients with S₃ disease, 7 developed S₁ disease, 3 maintained S₃ disease, and 1 worsened. In 7 patients with S₄ disease, 2 developed S₂ disease, 2 developed S₃ disease, 3 maintained S₄ disease (Table 2). One of two AT patients showed a notable regrowth of hair and achieved the S₂ disease (Fig. 1). In 14 AU patients, 10 patients exhibited hair regrowth, while the other 4 patients did not. At this time, the area of hair loss decreased to 25% or less of the whole scalp (S₀, S₁) in 7 patients (19.4%) out of 36 patients with the severe form of AA. Forty-five patients (20 male, 25 female) could be followed up for 1 year from the start of topical immunotherapy with SADBE (Table 3). Four patients dropped out of the study. Among them, 1 male patient could not continue topical immunotherapy due to a change of address. Of the 3 female patients, topical immunotherapy was discontinued in 2 patients due to pregnancy. The total response rate was 57.8% (5 CR; 11.1% and 21 PR; 46.7%). In all the patients whose symptoms improved in the 6 months follow up, the maintenance or improvement of the area of hair loss continued for 1 year after the start of topical immunotherapy. Among the patients with severe AA (n = 33), whose symptoms were S₃, S₄, AT, or AU at the start of treatment, 13 patients (39.3%) achieved S₀ or S₁ disease. One year later, the total response rate in the patients with severe alopecia was 51.5% in total (2 CR and 15 PR). One patient with AU, that had developed 25 years before, responded well to the treatment with SADBE and the symptoms improved from S₅B₂ to S₂ after 1 year of treatment (Fig. 2).

There were 16 pediatric patients (8 male, 8 female) whose age was 15 years or younger at the first consultation. Of these patients with AA, 4 patients were S₂, 4 patients were S₃, 3 patients were S₄, and 5 patients were AU (Table 4). The total response rate of the pediatric patients 1 year after the start of topical immunotherapy with SADBE was 50.0% (1 CR, 7 PR). In 12 pediatric patients with severe AA, the total response rate was 41.7% (no CR and 5 PR). Among the pediatric patients with severe AA, 2 patients (16.7%) achieved S₀ or S₁ 6 months later and 5 patients (41.7%) achieved S₀ or S₁ 1 year later. However, no pediatric AU patients' symptoms were improved by this treatment.

Table 2
Result of topical immunotherapy with SADBE in all patients after 6 months.

Severity before treatment	After 6 months						
	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅ (AT)	S ₅ B ₂ (AU)
S ₁ (n = 5)	2	3					
S ₂ (n = 8)	1	4	2	1			
S ₃ (n = 13)		7	2	3	1		
S ₄ (n = 7)			2	2	3		
S ₅ (AT) (n = 2)			1			1	
S ₅ B ₂ (AU) (n = 14)				1	8	1	4
Total (N = 49)	3	14	7	7	12	2	4

Table 1
Concomitant disorders and response in study patients.

Associated disease	Atopic dermatitis		Allergic rhinitis, asthma, urticaria		Autoimmune disease		Thyroid disease		Psoriasis		Vitiligo	
	before	after 1 year	before	after 1 year	before	after 1 year	Before	after 1 year	before	after 1 year	before	after 1 year
S ₀				2								
S ₁ (n = 5)		4	1	1	1	1		2				
S ₂ (n = 8)	2		1	1			1	1				
S ₃ (n = 13)	2		1		1		3					
S ₄ (n = 7)	2	3	1	1	1		1					
S ₅ (AT) (n = 2)												
S ₅ B ₂ (AU) (n = 14)	3	2	1		2	1			1	1	1	1
Total (N = 49)	9	9	5	5	5	2†	4	4	1	1	1	1

† Three patients discontinued topical immunotherapy.

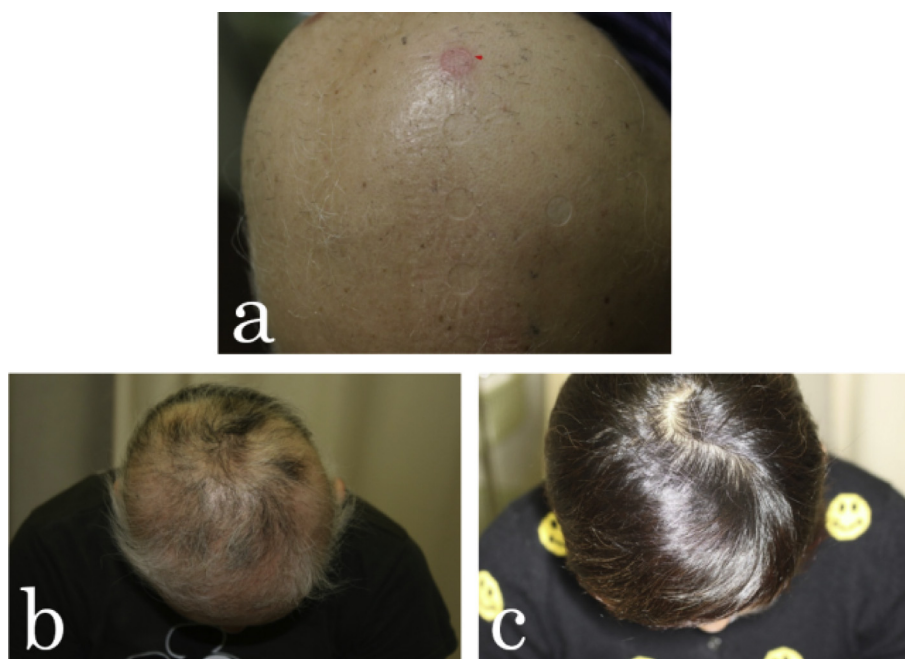


Fig. 1. Result of topical immunotherapy with SADBE in a patient with severe AA. Minimal concentration required to induce erythema was determined 48 h after the patch test (a), 3 months after the first application of SADBE (b), and 9 months after the first application of SADBE (c).

Table 3

Result of topical immunotherapy with SADBE in all patients after 12 months.

Severity before treatment	After 12 months						
	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅ (AT)	S ₅ B ₂ (AU)
S ₁ (n = 4)	2	2					
S ₂ (n = 8)	1	5	1	1			
S ₃ (n = 13)	1	8	1	2	1		
S ₄ (n = 6)		2	2	0	2		
S ₅ (AT) (n = 2)	1					1	
S ₅ B ₂ (AU) (n = 12)		1	1	1	7		2
Total (N = 45)	5	18	5	4	10	1	2

Thirty-three patients (13 male, 20 female) were 16 years or older at the first consultation. Among them, 4 patients dropped out of the study 1 year later (Table 5). Assessment of the 29 patients 1 year later revealed the total response rate in the adult patients to be 62.1% (4 CR, 14 PR). The achievement rates of S₀ or S₁ in the adult patients with severe AA, whose symptoms were S₃ or worse, were 5 of 24 patients (21%) after 6 month and 8 of 21 patients (38.0%). In the severe adult AA patients, the total response rate was 47.6% (2 CR, 10 PR) after 1 year of treatment.

Among 49 patients, 9 patients were complicated by AD (Table 1). Other complications were type 1 allergy disease (5 patients),

autoimmune disease (5 patients), thyroid disease (4 patients) and vitiligo (1 patient).

Discussion

Based on several case series studies and other studies, the efficacy of topical immunotherapy in the treatment of AA is estimated to be a response rate of about 50–60% (range from 9 to 87%).^{3–5} Our study showed similar results in terms of total response rate. There are reports that cases of AA that have developed at a young age or

Table 4

Result of topical immunotherapy with SADBE in pediatric† patients after 12 months.

Severity before treatment	After 12 months						
	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅ (AT)	S ₅ B ₂ (AU)
S ₁ (n = 0)							
S ₂ (n = 4)		1	2		1		
S ₃ (n = 4)			3				1
S ₄ (n = 3)			2	1			
S ₅ (AT) (n = 0)							
S ₅ B ₂ (AU) (n = 5)							5
Total (N = 16)	1	7	1	1			6

† 15 years or younger patients.

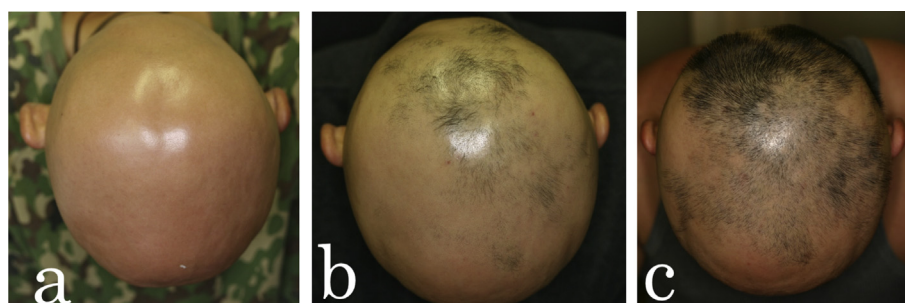


Fig. 2. Result treatment result of a severe AA patient (AU) before treatment (a), 6 months after treatment (b), and twelve months after treatment (c).

Table 5
Result of topical immunotherapy with SADBE in Adult† patients after 12 months.

Severity before treatment	After 12 months							
	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅ (AT)	S ₅ B ₂ (AU)	dropped out
S ₁ (n = 5)	2	2						1
S ₂ (n = 4)		3	1					
S ₃ (n = 9)	1	5	1	2				
S ₄ (n = 4)			1		2			1
S ₅ (AT) (n = 2)	1					1		
S ₅ B ₂ (AU) (n = 9)		1	1	1	2		2	2
Total (N = 33)	4	11	4	3	4	1	2	4

† 16 years or older patients.

have had a large area of hair loss are difficult to treat.⁶ Summarizing the cases we experienced (Table 6), the total response rate was lowest in the patients who were 16 years or older and had a severe form of alopecia, however, there was no statistically significant difference between groups. Tosti *et al.* followed up 191 patients with AA for a long period and reported that in adult patients, the higher the severity of AA at the time of onset, the worse their prognosis; this report supports our results.⁷

In our hospital, SADBE is used first in topical immunotherapy. When performing sensitization, a high concentration of SADBE is usually applied to a 4 cm × 4 cm circulation area using patch test tape.⁸ After sensitization has been achieved, we perform a patch test with a dilute concentration series of SADBE in order to quickly determine the appropriate concentration, and only then do we start the treatment at one-tenth of the minimal concentration to induce erythema. Because of this treatment strategy, no patient developed serious adverse side effects such as the formation of blisters or toxicoderma. While the strategy in which SADBE is applied to half of the scalp to determine the effectiveness is thought to be useful (the half-head method),^{9,10} we did not employ it in our study because not all the patients had bilateral symmetric alopecia. No randomized controlled trials have evaluated the effectiveness of topical immunotherapy in the treatment of AA¹¹; observational studies have used the half-head method to control for the spontaneous regrowth of hair.¹²

Although AD was the most frequent complication, a correlation with the severity of AA was not apparent. There have been many reports that AA is complicated by AD,^{13,14} and it is thought that atopic disposition is related to an increase of severity in AA.¹⁵ However, the exact mechanism of AA related complications is still not clear. In our study, there was no significant difference between the AA patients with and without AD in terms of the response to the immunotherapy (Table 1). Muller *et al.* reported that frequent complications with AA were thyroid disease (8%) and vitiligo vulgaris (4%).¹⁶ In our study, 4 cases of thyroid disease and one case of

vitiligo were noted, none of which required additional treatment for these complications. With regard to autoimmune diseases, 3 AA patients were excluded from the topical immunotherapy. Two of these patients required treatment for their autoimmune diseases by oral steroid therapy, and one wished to attempt to conceive children in the near future. Furthermore, our limited number of patients in our study, there was no evidence that the existence of complications affected the efficacy of immunotherapy (Table 1). However, because the pathogenesis of AA has yet to be fully elucidated, we believe that these complications should be investigated further to investigate the etiology of AA.

In conclusion, our results were in accordance with the previously reported effectiveness of topical immunotherapy for the treatment of AA. Although topical immunotherapy is effective, it does not always result in all patients achieving remission and some patients can experience a recurrence of AA after the cessation of treatment. In addition, topical immunotherapy is not covered by health insurance in Japan and thus this treatment is available only to a limited population of patients. As a result, most patients do not have access to this treatment. Furthermore, in some cases, the treatment has to be discontinued due to a change of address or a change in school.

Therefore, we aim to obtain more case data and continue to evaluate therapies other than topical immunotherapy for the treatment of AA. Furthermore, a novel treatment for AA is required to increase the number of patients with a sustained reduction in symptoms and to suppress disease recurrence in severe AA patients.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

KS, SF, and HI designed the study and wrote the manuscript. SM, MJ, TM and YI contributed to data collection. All authors read and approved the final manuscript.

References

- Tsuboi R, Itami S, Manabe M, Amo Y, Ito T, Inui S, et al. [Japanese Dermatological Association alopecia areata clinical practice guidelines 2017]. *Jpn J Dermatol* 2017;**127**:2741–62 (in Japanese).
- Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines-Part II. *J Am Acad Dermatol* 2004;**51**:440–7.
- Rokhsar CK, Shupack JL, Vafai JJ, Washenik K. Efficiency of topical sensitizers in the treatment of alopecia areata. *J Am Acad Dermatol* 1998;**39**:751–61.
- Price VH. Treatment of hair loss. *N Engl J Med* 1999;**341**:964–73.
- Dall'oglio F, Nasca MR, Musumeci ML, La Torre G, Ricciardi G, Potenza C, 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 Dermatol Treat* 2005;**16**:10–4.
- Wiseman MC, Shapiro J, MacDonald N, Lui H. Predictive model for immunotherapy of alopecia areata with diphencyprone. *Arch Dermatol* 2001;**137**:1063–8.
- Tosti A, Bellavista S, Iorizzo M. A long term follow-up study of 191 patients. *J Am Acad Dermatol* 2006;**55**:438–41.
- Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update Part II. *Treatment. J Am Acad Dermatol* 2010;**62**:191–202.
- Cotellessa C, Peris K, Caracciolo E, Morenti C, Chimenti S. The use of topical diphenylcyclopropenone for the treatment of extensive alopecia areata. *J Am Acad Dermatol* 2001;**44**:73–6.
- Aghaei S. Topical immunotherapy of severe alopecia areata with diphenylcyclopropenone (DPCP): experience in an Iranian population. *BMC Dermatol* 2005;**26**:5–6.
- Delamere FM, Sladden MM, Dobbins HM, Leonardi-Bee J. Interventions for alopecia areata. *Cochrane Database Syst Rev* 2008;**2**:CD004413.

Table 6
Total response rate of SADBE treatment after 12 months.

	Total response: CR + PR†(%)
All patients (n = 45)	57.8
All severe‡ patients (n = 33)	51.5
Pediatric§ patients (n = 16)	50.0
Severe pediatric patients (n = 12)	41.7
Adult patients (n = 29)	62.1
Severe adult patients (n = 21)	47.6

† CR, Disappearance of bald patches; PR, The hair regrowth in 50% or more of the hair loss area.

‡ 50% or more hair loss.

§ 15 years or younger patients.

12. Harries MJ, Sun J, Paus R, King LE. Management of alopecia areata. *BMJ* 2010;**341**:c3671.
13. Shellow WV, Edwards JE, Koo JY. Profile of alopecia areata : a questionnaire analysis of patient and family. *Int J Dermatol* 1992;**31**:186–9.
14. Katagiri K, Arakawa S, Hatano Y. In vivo levels of IL-4, IL-10, TGF-beta1 and IFN-gamma mRNA of the peripheral blood mononuclear cells in patients with alopecia areata in comparison to those in patients with atopic dermatitis. *Arch Dermatol Res* 2007;**298**:397–401.
15. De Weert J, Temmerman L, Kint A. Alopecia areata: a clinical study. *Dermatol* 1984;**168**:224–9.
16. Muller SA, Winkelmann RK. Alopecia areata, an evaluation of 736 patients. *Arch Dermatol* 1963;**88**:290–7.