Assessment of treatment efficacy of diphenylcyclopropenone (DPCP) for alopecia areata

Abstract

Background/aim: Alopecia areata is an inflammatory disease with a genetic and autoimmune basis. We aimed to study the efficacy, and safety of an immunomodulatory therapeutic agent, diphenylcyclopropenone, while manifesting the association with histopathological features, prognostic factors and side effects.

Materials and Methods: In this retrospective study, 98 patients (60 men, 38 women) with alopecia who were referred to the “Hair Disease Polyclinic” at Department of Dermatology between 2011 and 2015 were included. Together with medical histories and dermatological examinations, all patients were taken a skin biopsy for histopathological examination prior to the therapy. Therapeutic success was evaluated on the basis of hair re-growth percentage.

Results: Regarding the overall treatment success, 33 (34%) patients had complete response, 16 (16%) patients had partial response (between 50-99%), 27 (28%) patients had minimal response (between 1-49%), and 22 (22%) patients were non-responders. Both genders were equally represented in the outcome.

Conclusions: There was a significant relation between the severity of alopecia and treatment outcome. (p= 0.038) Patients with alopecia areata had significantly better response when compared to alopecia totalis and universalis. There was no statistically significant relation with other parameters such as disease duration, age, gender, atopy history, age of onset, and histopathological features.
1. Introduction

Alopecia areata (AA) is an inflammatory disease with a genetic and autoimmune basis, leading to various degrees of non-scarring patchy hair loss of the scalp and whole body areas. The estimated prevalence of alopecia areata is between 0.1% and 0.2% [1]. The exact aetiology of AA is still unknown, but genetic disposition has been identified, with some genes like TRAF1/C5 locus. Autoimmunity also plays a major role, with accompanying diseases of T cell auto-reactivity like Hashimoto’s thyroiditis or vitiligo. According to some studies, having a history of atopy also increases the risk of alopecia areata [2]. Cytokines like interferon-gamma, interleukins, and tumor necrosis factor alpha are thought to play a major role in the pathogenesis of the disease.

Clinically patients present with asymptomatic, patchy hair loss, with normal appearing underlying skin. Spontaneous resolution can occur, especially for those with a limited involvement. However many patients have a chronic and recurrent course, with many attacks over years. Treatment of alopecia has always been a big challenge for physicians. There are many different treatment modalities, with variable efficacies, including topical, intralesional or systemic corticosteroids, cyclosporine-A, local or systemic phototherapy, interferon-α, photodynamic therapy, acupuncture, topical minoxidil, anthralin and topical immunotherapy agents.

Topical application of diphenylcyclopropenone (DPCP) was originally developed in 1978 by Happle et al [3]. The exact mechanism of action of this treatment modality is not fully discovered. However DPCP induces an allergic contact dermatitis and this is thought to
decrease the T-cell-mediated immune reaction against the hair bulb. There is also evidence that it acts on the autoreactive T-lymphocytes within the follicular milieu to induce apoptosis. DPCP immunotherapy has a modulatory effect on the proinflammatory cytokines within the hair follicle and also provides antigenic competition, to distract the lymphocytes from their primary target.

In this study efficacy and safety of topical immunotherapy with DPCP have been evaluated retrospectively. Also the favorable prognostic factors to predict the response to DPCP immunotherapy were assessed.

2. Material and Methods

2.1. Patients and study design

In this retrospective study, 98 patients (60 men, 38 women) with alopecia areata, totalis or universalis who were referred to the “Hair Disease Polyclinic” at Department of Dermatology of Cerrahpaşa Medical Faculty, from August 2011 to June 2015 were included. All of the patients had clinically diagnosed alopecia and for all of them the diagnosis was confirmed with histopathological examination. The patients were resistant to any other topical or systemic conventional therapies for at least 6 months. Prior to the therapy patients were informed about the efficacy, estimated duration and possible side effects of the therapy such as enlargement of lymph nodes on the head and neck area, severe eczematous reactions like bullous eruptions or urticarial plaques limited to scalp or extending to other body parts, fever, malaise and fatigue especially within two days after the treatment and sometimes anaphylaxis-like reactions. All the patients gave their informed consent. If the patient is below 18 years old, consents of the families were taken. The mean age of the patients was 23.5, ranging from 5 to 59 years. Exclusion
criteria included current pregnancy or lactation, children under the age of 5, patients with vitiligo, severe photosensitivity or active systemic malignancies. Before the initiation of the therapy, factors such as gender, duration of the disease, age of onset, age at the beginning of therapy, previous treatments, history of atopy, type and severity of hair loss (in forms of percentage), presence of ophiasis pattern, eyebrow, eyelash, beard, and body hair involvement were recorded. On the first visit, a physical assessment was performed, with emphasis on grading the percentage of scalp involvement, type of alopecia and nail involvement. Prior to the therapy, all patients were taken a skin biopsy of 4 mm in diameter from the affected site on the scalp and histopathological investigations were made. The following parameters were assessed during histopathological investigations of the scalp biopsies; number of total follicular units, number of total hair follicles, number of follicular stelae, number of anagen, catagen and telogen hair follicles, the degree of lymphocytic infiltration of the hair follicle and degree of perifollicular fibrosis. The degree of fibrosis is evaluated numerically as 0; no fibrosis, 1; mild fibrosis, 2; moderate fibrosis and 3; severe fibrosis. Lymphocytic infiltration was also assessed similarly; as 0; no lymphocytic infiltration, 1; mild lymphocytic infiltration, which represents involvement of less than 10% of total number of hair follicular bulbus and presence of less than 3 rows of lymphocytes around each hair follicle, 2; moderate lymphocytic infiltration which represents involvement of 10-50% of hair follicular bulbus and presence of 3 to 6 rows of lymphocytes around each hair follicle and 3; severe lymphocytic infiltration, which represents involvement of more than 50% of all hair follicular bulbus and presence of more than 6 rows of lymphocytes around each hair follicle.
At the end of our study, therapeutic success was evaluated on the basis of percentage of hair re-growth on the scalp.

2.2. Treatment Method with DPCP

Topical immunotherapy with DPCP was performed following a standard protocol of sensitization. This protocol comprises the application of 2% concentration of DPCP solution diluted in acetone, over an area of 2 x 2 cm area on the occipital region of the scalp. The patients were told to avoid water contact of the sensitized area for two days and avoid sun exposure with the help of a wig or protective hat. After 2 days, patients were checked to detect whether or not sensitization to DPCP has occured. There is a lag period of two weeks after the sensitization, and then the treatment starts. The initial DPCP concentration is 0.001%. DPCP solution is applied to all affected areas on the scalp, together with eyebrows once in every week. Again after each session, patients were told to avoid water contact for 2 days, including excessive sweating and sun exposure. DPCP solution was left on the scalp for 48 hours and then washed off with a mild shampoo. The concentration of DPCP solutions were increased weekly, unless there were serious side effects including irritant contact dermatitis and photoallergic reactions, and the final concentration of 2% was reached at the end of sixth week. (Concentrations of 0.001%, 0.01%, 0.1%, 0.2%, 0.5%, 1% and 2% are applied sequentially.)

2.3. Follow-up and Assessment of Efficacy

During follow-up visits, the patients’ side effects were recorded, as well as the grade of hair re-growth. Once complete or cosmetically acceptable hair re-growth (amount of growth which eliminates the need of using wig or hat) was achieved, the intervals of the
DPCP application were prolonged to 2 weeks, 3 weeks and monthly. By this method, DPCP immunotherapy had been discontinued gradually. In case of hair loss during this tapering-off period, therapy was restored in weekly intervals. If there is no obvious response at the end of 6 months, immunotherapy was considered as non-effective and discontinued.

2.4. Statistical Analysis

Statistical analysis were performed using SPSS software programme (version 19.0, IBM Corp, Armonk, NY). Mann-Whitney, Wilcoxon and Kruskal-Wallis statistical tests were performed for each data set and a p value of less than 0.05 was considered as significant.

3. Results

The mean age of the patients was 23.2, ranging from 5 to 59 years. Mean age of male patients was found to be 21.8, with a standard deviation of 12.4. Mean age of female patients was found to be 25.5, with a standard deviation of 15.6. The demographic and clinical data are shown in Table 1. Mean period of disease was found as 4.2 years before DPCP treatment (Table 1).

Among 98 patients, 49 (50%) were diagnosed as alopecia areata, while 19 (19.4%) as alopecia totalis and 30 (30.6%) as alopecia universalis. At the beginning of the immunotherapy, 9 (9.25%) of the patients had a hair loss of below 25% (Alopecia Areata Investigational Assessment Guidelines- S1), 13 (13.25%) between 25 to 50% (S2), 16 (16.25%) between 50 to 75% (S3) and 11 (11.25%) patients between 75 to 99% (S4). Of
all the patients, half of them 49 (50%) had a total hair loss on the scalp area (S5), with a diagnosis of alopecia totalis or universalis.

20 (20.4%) patients had a previous history of atopy, including atopic dermatitis or allergic asthma.

With regard to the previous treatments, 37 (37.7%) patients had used only topical treatment modalities, including topical corticosteroids, minoxidil (2% or 5% concentration) and intralesional corticosteroid injections. 61 patients (62.3%) had tried both topical and systemic treatment options, including systemic corticosteroids, cyclosporine and ultraviolet therapy.

The mean duration of DPCP immunotherapy in the analysed cohort was 13.1 months, with a minimum of 4 months and a maximum of 44 months.

Regarding the overall treatment success 33 (34%) patients had complete response (Figure 1), 16 (16%) patients had partial response (between 50-99%) (Figure 2), 27 (28%) patients had minimal response (between 1-49%), and 22 (22%) patients were non-responders (Figure 3). Both genders were equally represented in the outcome.

Among 22 non-responding patients, 8 patients had 100% response and 7 patients had 75-99% response during the course of the therapy followed by a sudden and complete loss of hair.

Mann-Whitney U test was used to investigate the relation between type of the alopecia and treatment result. Statistical analysis revealed a significant relation between the type and severity of alopecia and treatment outcome. (p=0.038) Alopecia areata patients had
significantly better response to treatment, when compared to alopecia totalis or universalis. On the other hand, there was no statistically significant difference between success rate of the therapy and other parameters such as the disease duration before DPCP therapy, age, gender, atopy history, age of onset of alopecia, age at start of DPCP immunotherapy.

Histopathological parameters were also compared with the success rates of the treatment but there were no significant relationship considering the presence of fibrosis, lymphocytic infiltration, number of follicular stelae and number of catagen hair follicles (Table 2; P=0.478, 0.148, 0.994, and 0.118 respectively) (Figures 4a, 4b, 4c, 4d, 4e, 4f).

Total number of sessions and thus duration of therapy that a patients received were found to be positively and significantly correlated with the success rates, meaning the more sessions a patient receives and the longer the therapy continues, better the response would be.

During the sensitization process, almost all patients experienced minimal erythema, itching and burning sensation over the affected area. However, during the course of the therapy, among 98 patients, 56 (57%) showed no side effects. 38 (38%) patients complained about minimal erythema and sensitivity over the scalp, especially during the subsequent two days after the application. Other common side effects include pruritus, dermatitis, vesicles, bullae and flu-like symptoms. 4 (4%) patients had serious side effects like lymph node enlargement, fever and general malaise. 3 (3%) patients had irreversible hyperpigmentation of the head and neck area and 2 (2%) patients developed vitiligo macules.
4. Discussion

Alopecia areata is an organ-specific autoimmune disease, and the stem cells of the hair follicles are characteristically spared; which implies a potential for the regrowth of hair. Despite this fact, up to now there is no curative treatment for alopecia areata and none of the existing treatment options are able to alter the course of the disease. Phototherapy or photochemotherapy, topical and oral corticosteroids, cyclosporine and biological agents such as efalizumab and etanercept have all been used with various success rates [4-8]. Among all these treatment options, topical immunotherapy agents reveal promising clinical success with low side effect profiles. Topical sensitizers such as dinitrochlorobenzene, SADBE, or DPCP conduct an allergic contact dermatitis, via delayed-type (type IV) hypersensitivity reaction [9]. Dinitrochlorobenzene is highly mutagenic and squaric acid dibutylester can easily lose stability in case of long term use and high temperatures [10]. Thus, diphenylcyclopropenone is the choice of topical agent for this study, as well as many others in the literature [11] The exact mechanism of action for DPCP is still not clarified, however the likelihood of a regulatory-T lymphocyte mediated role within the follicular unit is high [12]. Although antigenic competition with the responsible antigens is by far the most accepted theory, other remarkable effects over the cytokine system include increased IL-10 secretion and low CD4⁺ to CD8⁺ T cell ratios, together with the induction of other immunomodulatory substances such as CTLA4 [13,14].

In many studies of DPCP topical immunotherapy, initially the solution is applied to one half of the scalp and only after there is hair growth, the treatment is extended to the entire scalp [15]. This approach aims to determine the efficiency of the treatment while
minimizing the side effects. However in our study, after sensitization process, solution is applied to all the hairless areas of the scalp, to obtain cosmetically acceptable results sooner.

Combination therapy with minoxidil or other agents did not improve the success rates according to many studies [16-18]. Therefore any topical or systemic treatment other than DPCP is not given to our patient group.

The studies reveal a wide range of treatment efficacies, rising up to 85% success rate for cosmetically acceptable hair regrowth. In a study of 148 patients, even 100% regrowth rate was achieved for patients with less than 50% hair loss [19-24]. This wide range of results could be due to the differences in treatment protocols, data sets, evaluation methodology, prognostic factors, absence of uniform terminology for evaluating results and the difference between statistical methods used for analyzing results. Some studies, on the other hand, showed no difference between topical immunotherapy and placebo or other treatment modalities such as topical corticosteroids, especially for patients with limited alopecia lesions [25,26]. These findings contradict with the previous data of the literature, as well as our study which showed clearly a better response in cases with limited involvement. According to the overall results of the studies in the literature DPCP immunotherapy for alopecia areata is a powerful option with limited side effect profile.

According to the previous efficacy studies of DPCP, chronic and extensive disease, and accompanying nail changes are reported to be poor prognostic factors [20] while limited involvement for a short time period, absence of nail changes, older age of onset, and a negative history of atopic dermatitis correlates with a more favorable prognosis [27,28].
In our case series, success rate and treatment response were associated with only the extent of the hair loss, thus type of alopecia. Patients with limited alopecia areata had better prognosis and good response to therapy while extensively involved alopecia totalis and universalis cases had worse prognosis and resistant course. Other parameters such as gender, duration of the disease, atopy history, age of onset of alopecia, age at start of DPCP immunotherapy had basically no effect on the prognosis of the disease during DPCP immunotherapy. Many other studies also support this finding, as the severity of alopecia being the sole important factor for the outcome of the treatment [21,22].

Many adverse events have been reported due to the use of DPCP, including eczematous reactions, urticaria, vitiligo, lymphadenopathy, hyperpigmentation or erythema multiforme-like reactions [18]. In our study during the sensitization process, almost all patients experienced minimal erythema, itching and burning sensation. Overall, most commonly encountered side effect was erythema and itching, followed by formation of papules, vesicles, bullae and flu-like symptoms. Other less frequent side effects were lymph node enlargement, fever, general malaise, irreversible hyperpigmentation of the head and neck area and vitiligo macules.

The use of DPCP in children is still a controversial area, although some studies have shown good results with acceptable side effect profiles [29]. In our study, children above 5 years of age are also included and treatment results or safety parameters were similar to adult patients.

Alopecia areata can have serious aesthetic and psychological consequences for patients due to unpredictable course consisting of spontaneous remissions and relapses. All
conventional treatment options have limited chance of success. Topical DPCP immunotherapy is a safe and effective alternative for patients with resistant disease and long-term results will provide more precise data about the success rate of this treatment modality.

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None.
References


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Italian Journal of Dermatology and Venereology 2014; 149 (1): 25-45. doi:
10.4103/ijt.ijt_99_17

9. Holzer AM, Kaplan LL, Lewis WR. Haptens as drugs: contact allergens are
powerful topical immunomodulators. Journal of Drugs in Dermatology 2006; 5
(5): 410-416.

10. Wilkerson MG, Connor TH, Henkin J, Wilkin JK, Matney TS. Assessment of
diphenylcyclopropenone for photochemically induced mutagenicity in the Ames
assay. Journal of American Academy of Dermatology 1987; 17(4): 606-611. doi:
10.1016/S0190-9622(87)70244-8


endothelial growth factor, apoptosis inhibitors (survivin and p16) and CCL27 in alopecia areata
before and after diphencyprone treatment: an immunohistochemical study. British Journal of

early and late phase cellular immune reactions in human skin. Journal of
Investigative Dermatology 2013; 133 (9): 159-190. doi: 10.1038/jid.2014.196


22. Durdu M, Özcan D, Baba M, Seçkin D. Efficacy and safety of
diphenylcyclopropenone alone or in combination with anthralin in the treatment
of chronic extensive alopecia areata: a retrospective case series. Journal of
American Academy of Dermatology 2015; 72 (4):640-650. doi:
10.1016/j.jaad.2015.01.008.

diphenylcyclopropenone for the treatment of extensive alopecia areata. Journal of
American Academy of Dermatology 2001; 44 (1): 73-76. doi: 10.1111/j.1365-
2230.2006.02256.x

immune privilege is linked to prevention of NK cell attack. Journal of
Investigative Dermatology 2008; 128 (5): 1196-1206. doi: 10.1038/sj.jid.5701183

25. Ito T, Ito N, Bettermann A, Tokura Y, Takigawa M. Collapse and restoration of MHC
class-I-dependent immune privilege: exploiting the human hair follicle as a model.
9440(10)63151-3


27. Weise K, Kretzschmar L, John SM, Hamm H. Topical immunotherapy in
alopecia areata: anamnestic for the treatment of extensive alopecia areata.
Journal of American Academy of Dermatology 1996; 192 (2): 129-133. doi:
10.1159/000246337

28. Iijima S, Otsuka F. Prognostic factors for clinical response of alopecia areata to
topical immunotherapy with squaric acid dibutylester. Archives of
Dermatology 1997; 133 (4):539-540. doi:
10.1001/archderm.1997.03890400145035

29. Donovan J, Salsberg J. The safety and efficacy of diphencyprone for the
treatment of alopecia areata in children. Archives of Dermatology 2012; 148:

Legends

Table Legends

Table 1: Demographic and clinical data of the patients

Table 2: The success rates and histopathological parameters with comparison to the type
of alopecia.

Figure Legends

Figure 1. (A) The appearance of the patient before DPCP, (B) full clinical response
after 4 months of DPCP treatment.
Figure 2. (A) The appearance of the patient before DPCP, (B) cosmetically acceptable response after 7 months of DPCP treatment.

Figure 3. (A) The appearance of the patient before DPCP, (B) patient not responding 6 months of DPCP treatment

Figure 4. (A) Mild fibrosis, HEX400 (B) Moderate degree of fibrosis, HEX400, (C) Severe fibrosis, HEX400, (D) Mild degree of lymphocyte infiltration, HEX400, (E) Moderate degree of lymphocyte infiltration, HEX400, (F) Intense lymphocyte infiltration, HEX400

Table 1. Demographic and clinical data

<table>
<thead>
<tr>
<th>Demographic data</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>98</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>M:60 (61%) / F:38 (39%)</td>
</tr>
<tr>
<td>Mean age</td>
<td>23.2 (Male:21.8, Female: 25.5)</td>
</tr>
<tr>
<td>Age at onset</td>
<td>Mean:28, Range:4-55</td>
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<tr>
<td>Disease duration</td>
<td>4.2 years</td>
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<tr>
<td>Previous local treatments</td>
<td>37 patients</td>
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<tr>
<td>Previous systemic treatments</td>
<td>61 patients</td>
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</tbody>
</table>
Mean treatment duration (months) 13.1
Mean treatment sessions 50.5

**Clinical data**

Type of alopecia

<table>
<thead>
<tr>
<th>Alopecia</th>
<th>Count</th>
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<tbody>
<tr>
<td>Alopecia areata</td>
<td>49</td>
</tr>
<tr>
<td>Alopecia totalis</td>
<td>19</td>
</tr>
<tr>
<td>Alopecia universalis</td>
<td>30</td>
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Table 2. The success rates and histopathological parameters with comparison to the type of alopecia.

<table>
<thead>
<tr>
<th>Type of Alopecia</th>
<th>Average Success Rate</th>
<th>P value</th>
<th>Average lymphocytic infiltration*</th>
<th>Average fibrosis**</th>
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<tbody>
<tr>
<td>Alopecia areata (0-25%)</td>
<td>74 %</td>
<td>0.012</td>
<td>2.7</td>
<td>1.4</td>
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<tr>
<td>Alopecia areata</td>
<td>70 %</td>
<td>0.022</td>
<td>1.5</td>
<td>2.4</td>
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<tr>
<td>Alopecia areata (25-50%)</td>
<td>67%</td>
<td>0.034</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>-------------------------</td>
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<td>-----</td>
</tr>
<tr>
<td>Alopecia areata (50-75%)</td>
<td>60%</td>
<td>0.040</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Alopecia areata (75-99%)</td>
<td>55%</td>
<td>0.045</td>
<td>2.1</td>
<td>2.0</td>
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<tr>
<td>Alopecia Totalis</td>
<td>53%</td>
<td>0.039</td>
<td>1.2</td>
<td>2.1</td>
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<tr>
<td>Alopecia Universalis</td>
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</tbody>
</table>

* Lymphocytic infiltration is valued as 0: no lymphocytic infiltration, 1: mild lymphocytic infiltration 2: moderate lymphocytic infiltration 3: severe lymphocytic infiltration

** Fibrosis is valued as 0: no fibrosis, 1: mild fibrosis 2: moderate fibrosis 3: severe fibrosis

Figure 1
Figure 2