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Topical Immunotherapy with Diphencyprone for in Transit and Cutaneously Metastatic Melanoma

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Topical diphencyprone (DPCP) can be used to treat in transit and cutaneously metastastatic melanoma. To date, 50 patients have received DPCP therapy for at least 1 month at our institution, with complete clearance of cutaneous disease in 46% and partial response in a further 38% of patients. Topical immunotherapy with DPCP is inexpensive and well-tolerated and should be considered in patients with skin metastases unsuitable for or refractory to other forms of therapy.
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KEY WORDS: contact hypersensitivity; diphenlycyclopropenone

INTRODUCTION

Melanoma is an immunogenic malignant tumor [1]. Histologic features of immune-mediated regression are frequently seen in primary lesions [2], and reduced or absent tumor-infiltrating lymphocytes in primary melanomas are independently associated with reduced disease-free survival [3,4]. Occasionally, spontaneous, and presumably immune-mediated regression of even widespread metastatic disease is observed [5], and a wide and increasing range of systemic therapies for melanoma act by harnessing and enhancing this immune sensitivity [1,6–8].

For patients with cutaneous recurrent melanoma, topical and intralesional immunotherapies have been used for more than 50 years [9–13]. Intralesional Bacille Calmette-Guerin has been reported to achieve local disease control in ~20% of patients [14], whilst the regression of untreated bystander lesions in patients receiving intralesional Rose Bengal suggests that immune-mediated regression is an important contributor to this agent's efficacy [15]. The contact sensitizer dinitrochlorobenzene (DNCB) has been used both intralesionally [16] and topically combined with systemic dacarbazine [17] for patients with in transit melanoma metastases. Overall lesion response rates of ~60–90% have been reported with DNCB used in this way [16,17].

Diphencyprone (DPCP), first synthesized in 1959 [18], is also a potent contact sensitizer which induces contact hypersensitivity (CHS) in ~98% of individuals [19]. Diphencyprone degrades on exposure to light, but when stored in UV-opaque containers in a cool, dark location it has a long shelf life of several months [20]. Unlike DNCB, DPCP has not been found to be mutagenic in the Ames assay [21,22], and is generally the preferred contact sensitizer for the treatment of cutaneous warts and alopecia areata [20].

In 1989, topical DPCP in combination with oral cimetidine was reported for the treatment of a patient with cutaneous metastatic melanoma [23], with another case report of its use in combination with dacarbazine and radiotherapy appearing in 2005 [24]. We first reported its use as a single agent for metastatic melanoma in 2007 [25–27] and now report the findings in our first 50 consecutive melanoma patients treated with topical DPCP at Melanoma Institute Australia.

MATERIALS AND METHODS

Diphencyprone Sensitization and Treatment Protocol

Adult patients with locally recurrent, in transit or cutaneously metastatic melanoma were offered treatment with topical DPCP if their disease was considered unsuitable for or refractory to conventional therapies such as surgery, radiotherapy, or regional or systemic chemotherapy. The use of DPCP in this setting was approved by the Ethics Committee of the Sydney Local Health District, and all patients provided written informed consent. Sensitization was performed using three drops of 2% DPCP in acetone (PCCA, Matraville, New South Wales, Australia) applied to the thin, sun-protected skin of the upper inner arm (protected from the immune suppressive effects of sun exposure [28]) on filter paper under Finn Chamber occlusion (Epitest, Tuusula, Finland) for 48 hr.

The time taken for development of contact sensitivity to epicutaneous antigens varies between individuals and also varies with antigen concentration, but is reported to occur ~8 to 15 days from the time of initial antigen application [29]. In our protocol DPCP diluted in aqueous cream was applied to melanoma lesions 10–14 days after sensitization. The use of an aqueous cream base allowed easier and more accurate application of DPCP than an acetone base. The initial concentration of

Abbreviations: CHS, contact hypersensitivity; DPCP, diphencyprone; DNCB, dinitrochlorobenzene.

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DPCP used at the site of melanoma metastases was generally 0.01–0.1%, applied unoccluded for 24 hr. In cases where recurrent disease affected a very large surface area of skin, DPCP was initially applied to an ~8–10 cm square test area. The resulting contact hypersensitivity (CHS) reaction was assessed 48–72 hr later at the time of peak CHS response [30]. The concentration of DPCP and its duration of application were then titrated over the course of subsequent applications to elicit moderate but tolerable contact dermatitis consisting of moderate erythema and mild induration, without blistering. DPCP was then re-applied once weekly for 2–24 hr, with the concentration and application time re-adjusted according to CHS intensity as required throughout treatment. Patients were instructed to maintain a "diphencyprone diary," recording the date, time and concentration of DPCP application, to facilitate this titration of CHS intensity.

In two patients the response to DPCP was very slow and so 5% topical imiquimod was added to the treatment regimen. Imiquimod was applied two to three times per week, including 24 hr prior to each weekly DPCP application.

RESULTS

Baseline Patient Characteristics

Fifty-eight patients commenced treatment with topical DPCP between May 2005 and December 2012; no patients were unable to be sensitized to DPCP. Of these, eight patients (four men and four women, mean age 67 years, range 41–90 years) did not complete at least 1 month of DPCP treatment; three patients because of rapidly progressing systemic disease, two patients because of bulky, rapidly progressing local disease, and three patients because they elected to stop DPCP after a single treatment because of pruritus or inconvenience. The demographic details of our 50 patients who completed at least 1 month of DPCP are given in Table I. More than half of these patients had disease of the lower limbs, and one patient had widespread dermal and epidermal melanoma metastases scattered over his scalp, face, hands and buttocks.

All 50 patients had extensive, biopsy-proven recurrent disease unable to be treated surgically. Forty-four had previously had surgical excision of recurrences with intent to achieve disease clearance, 16 had recurred despite previous radiotherapy and two each had failed previous intralesional Rose Bengal and systemic dacarbazine. None of our patients was considered suitable for further systemic therapy at the time of DPCP commencement. Thirty-one patients had thin epidermal or dermal metastases (with tumor deposits <5 mm thick), but 19 of our patients had bulky disease including cutaneous and subcutaneous lesions more than 5–10 mm thick.

DPCP Treatment Course and Followup

The concentration of DPCP used to achieve optimal CHS responses in these patients ranged from 0.00001% to 10%, with most patients (n = 40) needing concentrations between 0.1 and 0.0001%. Thirty patients developed blisters in the early stages of DPCP treatment, requiring dose reduction. Blistered lesions were managed with white soft paraffin applied under paraffin gauze dressings until spontaneous resolution 1–2 weeks later. Further DPCP, at a 10- to 100-fold lower concentration, was then reapplied once all blisters had healed and the erythema had faded. Four patients (all women) developed generalized dermatitis in addition to local blisters.

Dermatitis at distant sites from the treatment area was treated with topical 0.05% betamethasone dipropionate or 0.02% betamethasone valerate, with or without wet dressings. These cases of generalized dermatitis settled within 1–2 weeks and all the patients were able to continue DPCP at lower concentrations without recurrence of generalized dermatitis. One man developed generalized urticaria during DPCP treatment. This was controlled with oral loratidine 10 mg twice daily, and DPCP treatment was able to be continued. A 43-year-old woman with partial response to DPCP developed depigmentation at DPCP treated sites on her lower leg. Postinflammatory hyperpigmentation was frequent but temporary at treated sites in patients with skin type 3 [31]. The duration of DPCP treatment in our 50 patients ranged from 1 to 60 months (mean 15 months), and thus far the mean duration of followup is 20 months (range 1–78).

Diphencyprone Treatment Response

Overall, 23 patients (46%) achieved complete clearance of their cutaneous melanoma metastases (Table II and Figs. 1–4). An additional 19 patients (38%) showed a partial response to DPCP, with reduction or resolution of some but not all recurrent disease, and/or significant slowing of the rate of disease progression. Nine patients (18%) had no response to DPCP. There was no apparent influence of body site on treatment response; complete responses were observed in 46% of head and neck disease, 50% of truncal disease and 45% of lower limb disease. The mean age of the 23 complete responders was 74 years (range 46–89); the mean age of the 9 non-responders was 67 years (38–84). There was no apparent correlation between the intensity of early DPCP responses (i.e., presence or absence of blistering early in treatment) and complete response. Of the complete responders, 11 had blistering reactions and 10 had milder responses, and of the non-responders 4 had blistering and 5 had milder responses with erythema but no blistering.

In the two patients receiving concurrent imiquimod treatment, melanoma regression was accelerated; an 82-year-old man went on to have a complete response, including regression of metastatically involved lymph nodes, and a 43-year-old woman showed clearance of most but not all of the multiple metastases at her left lower leg.

All but five of our complete responders elected to continue DPCP after clearance of their disease, generally as a mild treatment once every 1–4 weeks. Despite this maintenance therapy, six complete responders later developed cutaneous recurrences which were again able to be cleared with more intensive DPCP. One complete responder with bulky subcutaneous disease as well as dermal metastases had used DPCP for 6 months with only partial response. He then began applying 5% imiquimod cream thrice weekly, including an application 24 hr before each of his weekly DPCP treatments. His disease then began to slowly regress and he is now disease-free (Fig. 5).

TABLE I. Demographic Details of 50 Patients to Complete at Least 1 Month of Topical Diphencyprone Therapy

Men/women	32:18
Mean age (range)	71 years (38–89)
Site of DPCP-treated disease	
Head and neck	13
Arm	2
Trunk	5
Lower limb/groin	29
Generalized	1

DPCP, diphencyprone.

TABLE II. Complete Responder Details

Men/women	16:7
Mean age (range)	74 years (46–89)
Mean DPCP treatment duration to clearance of all cutaneous lesions (range)	8 months (1–24)
Mean duration of complete response (range)	17 months (1–78)

Twenty-three patients had complete clearance of their cutaneous metastases with diphencyprone.
DPCP, diphencyprone.

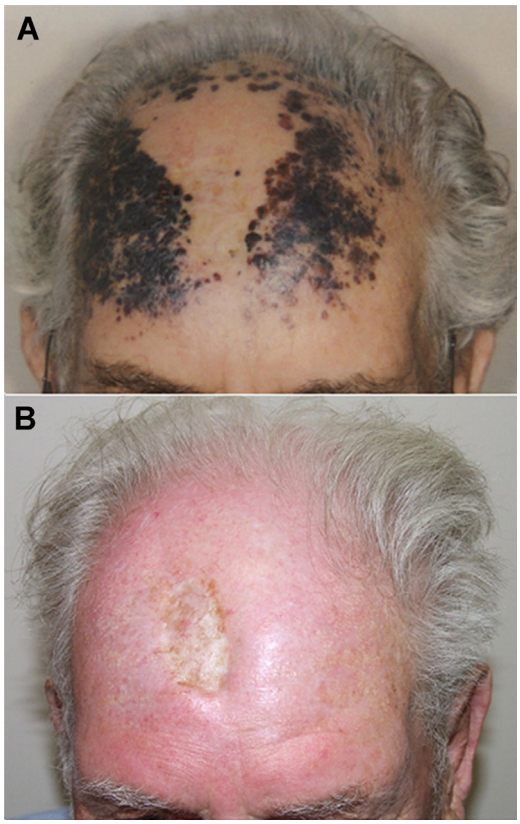

Fig. 1. Our first patient to receive topical DPCP for melanoma was a 71-year-old man with extensive, radiation-resistant recurrent scalp disease, shown prior to commencement of DPCP in May 2005 (**A**). Regression of all cutaneous melanoma occurred within 4 weeks of starting DPCP, and biopsy at the time of DPCP treatment showed an intense mixed inflammatory cell infiltrate comprising CD4+ and CD8+ T cells, CD79a+ B cells and also CD68+ macrophages. Five years later he was disease free (**B**).

As expected, patients with thin dermal or epidermal disease responded better than patients with bulky cutaneous and subcutaneous disease. Whilst 61% of patients with thin disease showed complete responses, and only 7% failed to respond at all, patients with bulky disease had a complete response rate of 21%, and 37% of these patients had no response to DPCP.

Eleven of our complete responders (48%) are currently disease-free, 2 and 3 are alive with cutaneous and systemic metastases respectively, and 6 have died with visceral metastases. One 87-year-old man died following a cerebrovascular accident, with no evidence of recurrent disease.

Regression of Lymph Node and Visceral Metastases

None of our patients had known lymph node metastases at the time of commencement of DPCP therapy. Four DPCP-treated patients had regression of clinically palpable metastatic lymph nodes which became evident during DPCP treatment. An 82-year-old man with dermal and subcutaneous melanoma deposits on the right cheek and neck had been treated with DPCP with partial response for 12 months when he developed a bulky submandibular nodal metastasis, which was confirmed by fine needle aspiration biopsy. A limited neck dissection was planned, but the nodal lesion resolved over the next 2–3 weeks and surgery was not required.

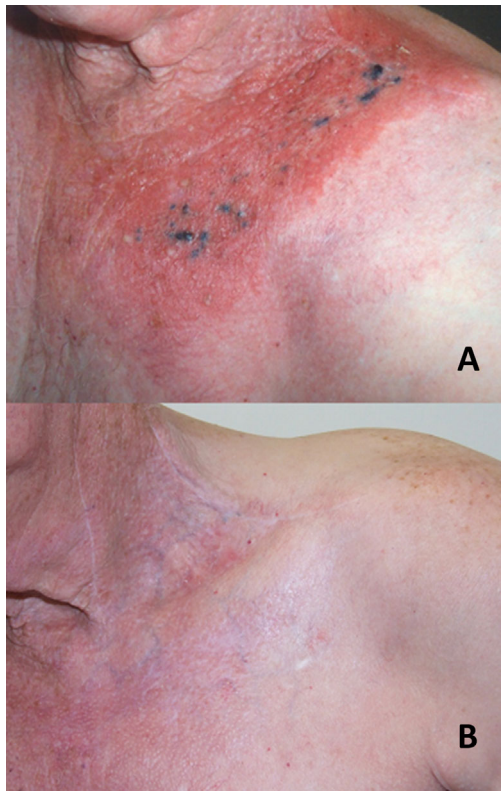

Fig. 2. A 72-year-old man with in transit melanoma metastases on the upper chest shows an ideal level of DPCP contact hypersensitivity, with intense erythema and mild local induration at the site of DPCP application (**A**). 6½ years later, he remains clinically and radiologically disease free (**B**).

An 85-year-old man treated with DPCP for bulky disease on his left face and neck developed extensive mediastinal node involvement, demonstrated on computed tomography, which was no longer apparent on repeat scanning 11 months later. A 72-year-old man treated with

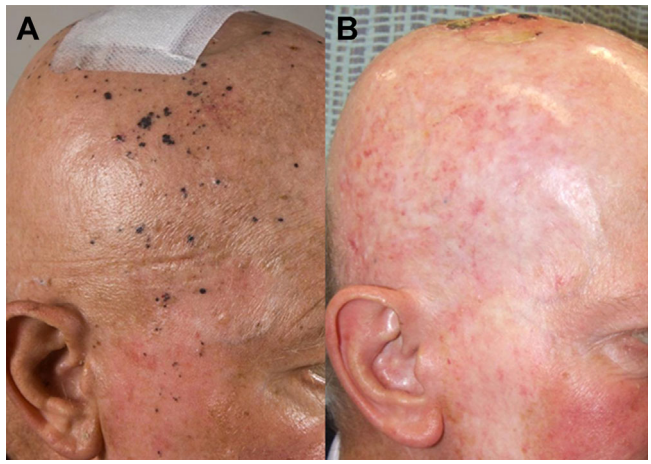

Fig. 3. A 69-year-old man developed extensive recurrent melanoma on his scalp, despite previous radiotherapy (**A**). Within 4 months of commencing DPCP treatment his metastases had resolved, and he remains disease free 4 years later (**B**).

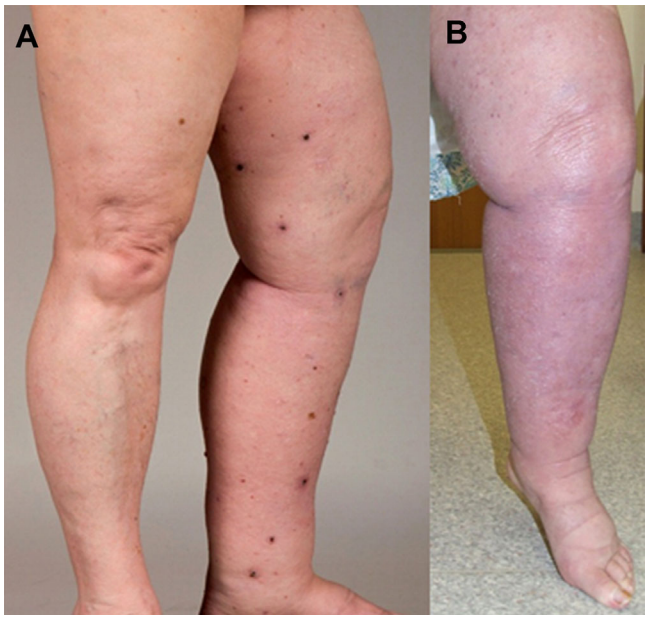

Fig. 4. Nodular *in transit* disease (**A**) in this 72-year-old woman cleared within 3 months of commencing DPCP (**B**).

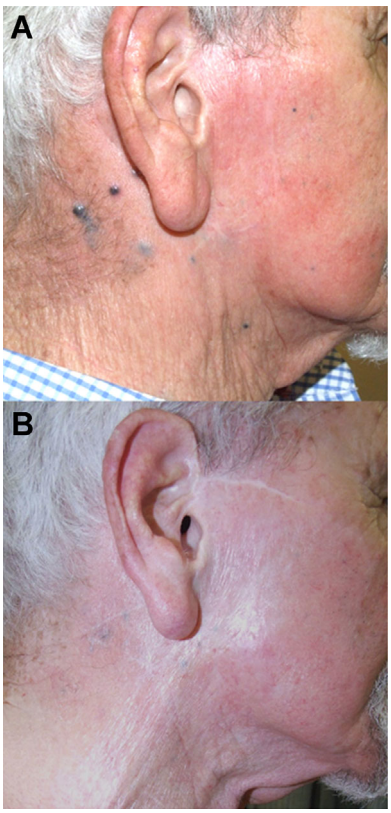

Fig. 5. An 82-year-old man with radiation-resistant recurrent cutaneous and subcutaneous melanoma at the right cheek and neck (**A**) has shown a complete response to DPCP in combination with topical imiquimod. He also showed regression of a histologically confirmed submandibular nodal metastasis, and remains disease free 3 years after commencing DPCP (**B**).

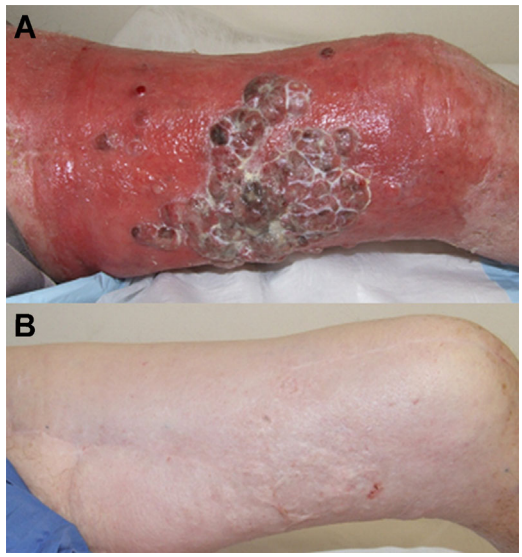

Fig. 6. An 82-year-old man initially failed to respond to DPCP, and developed fungating recurrent disease in the thigh (**A**), as well as bulky bilateral inguinal node disease and pulmonary metastases. Within 2 months his cutaneous, nodal and visceral disease regressed and he remains well 4 years later (**B**).

DPCP for dermal metastases on his left upper chest developed a 1.5 cm diameter hard left lower cervical mass, clinically consistent with metastatic disease. This completely resolved over the next 6 months and this patient remains disease free 6 years later. In one patient, an 82-year-old man treated with DPCP for fungating disease on his left thigh, there was regression of both his bulky bilateral inguinal node metastases, and also of his multiple pulmonary metastases, as seen with computed tomography and positron emission tomography [27]. He remains clinically and radiologically disease free 4 years later (Fig. 6).

DISCUSSION

Diphencyprone is frequently used to treat alopecia areata and cutaneous warts [20]. Potential side effects of treatment with topical contact sensitizers include a more intense than desired CHS response, with blistering, regional lymphadenopathy, and occasional generalized eczema or urticaria [20,32]. The risk of an excessive CHS response is generally proportional to the concentration and duration of DPCP application; in the treatment of benign skin conditions it is appropriate to commence with low concentrations of DPCP and gradually increase concentration until a mild to moderate response is achieved. In patients with progressing metastatic or recurrent melanoma in the skin, it is important to achieve a moderate CHS response promptly, so higher starting concentrations of DPCP may be used in order to minimize treatment delay. Our patients tolerated their CHS reactions well, requiring only white soft paraffin and dressings whilst the CHS reactions spontaneously resolved. Inflammation in lower legs, especially where there is venous disease and/or lymphedema from previous surgery, is much slower to settle, and so lower starting concentrations of DPCP (e.g., 0.01%) may be more appropriate in this setting. Similarly, frail elderly patients may be better able to manage DPCP treatment with a more conservative choice of starting concentration. Post-inflammatory hyperpigmentation and immune-mediated depigmentation have also been reported [20], although we observed the latter in only one of our patients.

Diphencyprone is presumed to act by promoting lymphocyte-mediated tumor destruction [33], although its exact mechanisms of

action in melanoma have not yet been determined. When used to treat cutaneous warts, DPCP was shown in one patient to cause predominance of $CD8^+$ cells, with reversal of the $CD4^+/CD8^+$ ratio, in the lesional inflammatory infiltrate [34]. We found a mixed infiltrate heavy in $CD4^+$ and $CD8^+$ cells as well as B cells and macrophages in our first DPCP patient. Reverse-transcriptase PCR of RNA extracted from paraffin embedded tissue in this patient showed modulation of the interleukin (IL)17 pathway in post-treatment compared to pre-treatment biopsy material [35]. TH17 lymphocytes have also been implicated in melanoma regression in B16 mice [36].

The topical immune-response modifier imiquimod has both antiviral and antitumor TH1 effects, mediated via activation of Toll-like receptor (TLR)-7 and TLR-8 and secretion of various cytokines including interferon (IFN)α, IFNγ, tumor necrosis factor, IL-1, IL-6, IL-8, IL-10 and IL-12 [37], and possibly via TLR 7 independent inflammation [38]. It has previously been used to treat cutaneous metastatic melanoma [39], although we have found it to be less effective in the treatment of extensive recurrent melanoma than DPCP [26]. The addition of imiquimod to our DPCP regimen, however, did seem to assist disease response in one patient with very slowly responsive metastases. Imiquimod may be a useful additional treatment to augment the DPCP CHS response in refractory cases.

Systemic agents might also augment the response to DPCP. The combination of DNCB and fotemustine has been reported as effective [40], and response rates of 37–62% have been reported with the concurrent use of DNCB and systemic dacarbazine in patients with stage 3 disease [17,41]. This may represent a synergistic response to these systemic and topical agents; in mice neither DNCB nor dacarbazine were effective in reducing subcutaneous melanoma deposits, whereas tumor growth was significantly reduced when the two agents were used in combination [42].

Topical therapy with DPCP in a cream base is easy for patients to apply, is well-tolerated and costs less than AU$1 per week. In a group where 40% of patients had bulky cutaneous and subcutaneous disease, we found complete clearance of skin disease in 46% of the total patient group in this unselected case series. In patients with thinner disease, more than 90% of patients had complete or partial responses to DPCP. We conclude that DPCP therapy provides an effective and inexpensive treatment option in melanoma patients with difficult cutaneous metastases, which are unsuitable for other therapies, and further studies are now warranted to determine its mechanisms of action.

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