Topical Immunotherapy of Basal Cell Carcinomas with Dinitrochlorobenzene


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SUMMARY

One hundred thirteen tumors in 5 dinitrochlorobenzene (DNCB)-sensitized patients with multiple basal cell carcinomas were treated with topical DNCB. Thirty-six of the 113, or 32%, showed complete clinical regression, for periods of 5 to 18 months. An additional 29% (33 or 113) showed partial regression. DNCB induced only 15 to 25% complete regression in 4 of the 5 patients, whereas 21 of 30, or 70% of basal cell carcinomas treated with DNCB underwent complete regression in the 5th patient. In addition to variation of patient response, there was variation of response of tumors on the same patient. Photographic data presented separately are of a 6th patient with uncountable confluent basal cell carcinomas who responded well to DNCB, but in whom accurate quantification on an individual tumor basis was not possible.

Controls in this study included 63 untreated tumors that were adjacent to and distant from treated tumors. These control tumors showed no evidence of regression. Eleven tumors treated with inert vehicles did not respond. One subject with over 100 nodular basal cell carcinomas became tolerant to DNCB and failed to respond to high concentrations of the drug. Croton oil, a primary irritant that induces inflammation without evidence of cell-mediated immunity, did cause complete regression in 6 of 26 (23%) treated tumors.

The mechanism of DNCB-induced regression of basal cell carcinoma remains unknown. The disappearance of locally invasive human cancer following the therapeutic imposition of a controlled allergic contact dermatitis provides a useful model for the study of immunotherapy of cancer in humans.

INTRODUCTION

Basal cell carcinoma is the most common cancer in humans (2). Basal cell carcinomas offer certain advantages for controlled, quantitative clinical studies of topical therapeutic agents. They usually present as discrete lesions that facilitate direct observation and accurate quantification of any therapeutic effect and, while they produce considerable morbidity by local invasion, they rarely metastasize (14). Furthermore, in contrast to many cancers, long-term follow-up is possible by selection for study of those patients with basal cell carcinomas who are otherwise in good health.

Conventional means of therapy such as excision, electrodesiccation, curettage, radiotherapy, cryotherapy, and chemotherapy all have produced cure rates approaching 100% (9). In patients with the basal cell nevus syndrome, xeroderma pigmentosum, arsenical dermatitis, or extensive radiation dermatitis, and in fair-skinned patients with severely actinically damaged skin, basal cell carcinomas may be so numerous that conventional therapy becomes impractical. Topical chemotherapy is often the treatment of choice for these patients.

The contact sensitizer DNCB\(^1\) has been used in the treatment of multiple basal cell carcinomas. Klein (8) reported that DNCB produced resolution of more than 95% of treated superficial basal cell carcinomas without recurrences for periods up to 5 years. Stjernswärd and Levin (15) reported that, in 8 of 9 patients treated with topical DNCB, at least 1 basal cell carcinoma disappeared, with no recurrence for 12 to 30 months. It seemed important to extend these studies by quantifying the observed therapeutic responses on an individual tumor basis. In this study we report the quantitative results of treatment of basal cell carcinomas with DNCB in 7 patients with multiple basal cell carcinomas.

MATERIALS AND METHODS

Patient and Tumor Selection. The study group consisted of patients referred to the NIH with multiple basal cell carcinomas, who remained hospitalized during initial therapy and returned at 1- to 2-month intervals for evaluation. Posttreatment duration ranges from 5 to 18 months. Patients with tumors predominantly on the face near the eyes, nose, and ears were excluded, and tumors in these locations on study patients were treated with conventional therapy.

Seven patients with 25 to more than 100 basal cell carcinomas were selected (Table 1). Four had the dominantly inherited basal cell nevus syndrome, and 3 had multiple basal cell carcinomas of unknown etiology, although excessive sun exposure played a role in 1 fair-skinned Texan (Patient 3) who exhibited actinic keratoses and solar elastosis in addition to his numerous basal cell carcinomas. None of the 7 patients was known to have been exposed to arsenicals.

Patient Evaluation. Patient evaluation included a complete history and physical examination, chest and skull X-rays, electrocardiogram, urinalysis, complete blood count, liver function tests, blood urea nitrogen, and creatinine assays. The blood and urine tests were obtained on each admission and the other tests were repeated when clinically indicated. All 7

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\(^1\) The abbreviation used is: DNCB, 1-chloro-2,4-dinitrobenzene.
patients had normal lymphocyte counts, no history of persistent or severe infection, and all could be sensitized to DNCB. Skin testing with microbial antigens was not performed in order to avoid additional artificial immunological stimuli.

**Tumor Evaluation.** All tumors were recorded on anatomical figure charts noting their location, size, and character (nodular, superficial) and were photographed before treatment. In each patient, 5 to 15 representative tumors were biopsied with a 3-mm punch for histological confirmation. Biopsied tumors less than 5 mm in size were excluded from the study. Larger tumors were included in the study after the biopsy site had healed. All tumors selected for treatment were over 2 mm in diameter.

**Sensitization to DNCB.** A sensitizing dose of 2000 μg DNCB (Eastman Kodak Co., Rochester, N. Y.) in 0.1 ml acetone was applied to the skin of the medial aspect of the upper arm. The DNCB was allowed to evaporate, and covered by an adhesive bandage for 1 week (1, 3, 4).

**Evaluation of Degree of DNCB Sensitivity.** Dilutional patch tests with DNCB in acetone or in cream [Acid Mantle Creme (aluminum acetate), Dome Laboratories, West Haven, Conn.] were performed 2 to 3 weeks after initial sensitization, except for Patient 6 who was patch tested 8 months after sensitization. Occlusive patch testing (Johnson & Johnson Sheer Patches) was performed on nondiseased skin with the use of approximately 50 mg of cream containing 10-fold differing concentrations of DNCB ranging from \(10^{-1}\) to \(10^{-6}\) g/100 g (w/w). After 48 hr, the patches were removed and the reaction was evaluated clinically as to macular erythema, erythema and induration, vesiculation, or bulla formation. Patch testing in some of the patients enrolled in the study was also performed by evaporating 0.1 ml of acetone containing half-log dilutions of DNCB ranging from 100 to 0.1 μg in 2-cm polyethylene rings on uninvolved skin and evaluating for the same criteria at 48 hr, both with and without occlusion. The minimum amount of DNCB eliciting a positive reaction was approximately the same on a given patient, with either the cream or acetone vehicle, although occlusive patch testing resulted in reactivity at 1 log dilution lower than an open patch test.

**Method of Treatment.** Tumors selected for treatment were challenged daily with the lowest concentration of DNCB showing erythema and induration on dilutional occlusive patch testing. The DNCB in either acetone or cream was applied directly to the tumors and a halo of surrounding normal-appearing skin and then occluded. This procedure, which was repeated once daily for a period of 8 weeks, constituted 1 course of therapy. If a tumor failed to respond, or responded poorly, additional courses of therapy were given. All 6 patients who remained sensitive to DNCB were given at least 1 course of DNCB in cream, and all patients received 16 to 24 weeks of therapy in 8-week courses with a range of 2 to 19 weeks between courses.

**Control Tumors.** (a) A total of 74 tumors in the 6 sensitive patients were not treated with DNCB for evaluation of possible distant effects of the DNCB therapy. To test the vehicles for possible effect, 4 of these tumors in Patient 4 were treated with acetone alone and 7 of these tumors in Patient 5 were treated with the cream vehicle alone. (b) One patient with acquired tolerance to DNCB had more than 100 nodular basal cell carcinomas and was treated with high concentrations of DNCB (see “Results”). This unresponsive patient serves as a control for the direct effect of DNCB on basal cell carcinomas. (c) Dilutional patch tests from 0.1 to 50% croton oil were performed. Twenty-six tumors were treated with 0.5% croton oil (Sigma Chemical Co. St. Louis, Mo.) in mineral oil once daily for 8 weeks with occlusion.

**Evaluation of Treated and Control Tumors.** Tumors were observed daily for the 1st 2 to 3 weeks of therapy for evidence of inflammation or tumor necrosis. Thereafter the tumors were examined, recorded on anatomical diagrams, and photographed during the 4th or 5th week of therapy and at 1- to 2-month intervals after a therapeutic course of DNCB.

The results indicate the cumulative effect of DNCB therapy on treated and control tumors and were obtained by comparing photographs taken prior to DNCB sensitization with photographs taken on the patients’ last hospitalization.

On the basis of these comparisons, tumors were scored as follows: (a) complete regression, defined as no clinical evidence of remaining tumor (e.g., superficial basal cell carcinoma in Figs. 1 and 2); (b) partial regression (e.g., Figs. 3 and 4); (c) no significant change (e.g., nodular basal cell carcinoma in Figs. 1 and 2); or (d) increased in size (Figs. 5 and 6). Tumors were scored as no significant change unless there was unanimous opinion among 4 observers that a significant increase or decrease in size had taken place. Partial regression included tumors with obvious volume change as well as tumors that were smaller by measurements in 2 dimensions.

**RESULTS**

**DNCB-treated Basal Cell Carcinomas.** Table 2 summarizes the results of DNCB treatment of 113 basal cell carcinomas in 5 patients with discrete lesions. Thirty-six of 113 or 32% of these carcinomas showed complete regression; an additional 33 of 113 or 29% of the tumors decreased in size. It should be noted that 4 of the 5 patients showed only 15 to 25% complete regression (Table 2), whereas Patient 4 showed 21 of 30 or 70% complete regression. In addition to variation in response among the patients, there was considerable variation in response of tumors even on the same patient. Complete regression of some tumors was seen while adjacent tumors showed no change (Figs. 1 and 2) or even increased in size.
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(Figs. 5 and 6). Eighty-four of the 113 tumors treated with DNCB were nodular. Response according to tumor size was evaluated in these 84 treated tumors. Thirty of the 75 nodular tumors between 2 and 5 mm in diameter showed complete regression, whereas none of the 9 nodular tumors greater than 5 mm in diameter showed complete regression. Six of these 9 nodular tumors increased in size and the other 3 showed partial regression.

Patient 6 had multiple superficial basal cell carcinomas predominantly over his lower back (Figs. 7 and 9) and showed a good response to DNCB challenge (Figs. 8 and 10). However, the great number of confluent tumors in this patient made it impossible to accurately estimate the regression rate for individual lesions.

The results of DNCB patch tests showed that DNCB sensitivity varied during the course of the study for individual patients. Patient 7 became tolerant to DNCB while Patients 2 and 4 developed a 2 log increase in sensitivity. Patient 4 was the most sensitive patient, showing erythema and induration to $10^{-3}$ g/100 g DNCB in cream. The 6 patients were treated with DNCB in concentrations ranging from $10^{-4}$ to $10^{-2}$ g/100 g.

Control Basal Cell Carcinomas. None of 63 untreated tumors, or of 11 additional tumors treated with inert vehicles, showed any regression (Table 3).

Patient 7 was initially sensitive to DNCB showing erythema and induration at $10^{-3}$ g/100 ml in acetone and was the only subject initially treated with a suberythema concentration of DNCB. He demonstrated mild erythema of normal skin and tumors after 10 days of daily application of DNCB, $10^{-4}$ g/100 ml, in acetone, but there was no evidence of tumor necrosis. The subject was then treated with higher concentrations of DNCB ($10^{-2}$ g/100 ml), resulting in a transient oozing and crusting of both normal skin and tumors. This reaction subsided after a few days with no evidence of tumor necrosis. The subject was then treated with higher concentrations of DNCB, had no response to croton oil in 2 tumors.

8-week course of therapy, and no tumor necrosis or appreciable change in his tumors occurred. The inflammatory response produced by DNCB in this subject was not as severe or sustained as that produced by the daily application of 10% croton oil in other subjects.

Twenty-six tumors in 3 patients were treated with croton oil (7, 11), a primary irritant that is not reported to induce cell-mediated allergic contact dermatitis (Table 2, Line 2). Two tumors in Patient 4, the best DNCB responder, did not decrease in size after 8 weeks of daily application of 10% croton oil. Patient 2 showed complete regression of 1 superficial basal cell carcinoma treated with 10% croton oil. Twenty-three of 26 croton oil-treated tumors were in Patient 3 who was a poor DNCB responder. In this patient, 5 of 23 tumors treated with croton oil and 3 of 19 treated with DNCB showed complete regression. Results of dilutional patch tests with croton oil on these 3 patients showed that 10% croton oil induced an inflammatory response, whereas 1% croton oil did not.

Complications of DNCB Therapy. No severe complications due to DNCB challenge were noted in any of the 7 subjects. Patient 2 developed a transient sterile eosinophilic pleural effusion during a course of DNCB therapy which subsided despite continued DNCB treatment. Liver function tests, complete blood counts, urinalysis, blood urea nitrogen, and creatinine tests were normal throughout the study in all 7 subjects. Patient 5 developed mild postinflammatory hyperpigmentation in many DNCB-treated sites. Patient 2 had bilateral varicosities with mild stasis dermatitis, and DNCB treatment resulted in hyperpigmentation predominantly in treated sites on his lower extremities. Patient acceptance of repeated challenge with DNCB was quite good. Pruritus from the allergic contact dermatitis was never so severe that therapy had to be discontinued.

**DISCUSSION**

In this study the 32% complete clinical regression was based on 5 subjects with discrete countable lesions, was limited to basal cell carcinomas more than 2 mm in diameter, and included superficial and nodular lesions. There was a marked variation of response among patients and among tumors on the same patient. The 32% regression rate should not be taken as a

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**Table 2**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Complete</th>
<th>Partial</th>
<th>Total treated</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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<td>3</td>
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</tr>
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<td>5</td>
<td>6 (6)</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>36 (30)</td>
<td>32</td>
<td>113 (84)</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses, number of nodular tumors included in category.

**Table 3**

<table>
<thead>
<tr>
<th>Type of control</th>
<th>Complete</th>
<th>Partial</th>
<th>Total included</th>
</tr>
</thead>
<tbody>
<tr>
<td>No therapy</td>
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<td>0</td>
<td>0 (0)</td>
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<tr>
<td>Croton oil</td>
<td>6 (5)</td>
<td>13 (13)</td>
<td>26 (20)</td>
</tr>
<tr>
<td>Acid mantle cream</td>
<td>0</td>
<td>0</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Acetone</td>
<td>0</td>
<td>0</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Tolerant patient</td>
<td>0</td>
<td>0</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

*b Twenty-three of the 26 croton oil tumors were in Patient 3. One complete regression was in Patient 2. Patient 4, the best responder to DNCB, had no response to croton oil in 2 tumors.

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final cure rate, since no attempt was made to demonstrate histological cure, and the maximum follow-up was 18 months. This figure of 32% does not include data from 1 subject who showed a good response but who had uncountable confluent basal cell carcinomas. This subject improved considerably, but because he did not have discrete countable lesions, accurate quantitation of individual tumor regression was not possible.

Klein (8) reported complete disappearance of over 95% of treated superficial basal cell carcinomas but did not stipulate size of tumors nor quantify individual tumor response. Klein sometimes used suberythema-inducing concentrations, as determined by dilutional patch tests, but as in this study, he usually employed the lowest concentration to which a reaction occurred on normal skin. We used a suberythema-inducing concentration only in the 1 subject who subsequently became tolerant. There were some differences in techniques between the study of Klein and this study. Subjects in this study were sensitized by the application of 1 large dose (2000 µg) of DNCB to the inner aspect of the arm, which was the method also used by Stjernswärd and Levin (15). Klein sensitized his subjects by the daily application of a lower concentration of DNCB to the forearm. We selected the single large dose method of sensitization because application of small quantities at weekly intervals to the forearm has been reported to result in a lower degree of sensitivity and even specific tolerance to DNCB (12). While daily spacing may result in a better degree of sensitization than is produced by weekly spacing, sensitization with a single dose of 2000 µg is a powerful method which results in sensitization of more than 96% of healthy subjects (3) and of a significant number of patients with Hodgkin’s disease (1).

The sporadic and inconsistent response of individual tumors to DNCB therapy may be related to size, morphological type, or other biological variables rather than to the intensity of the inflammatory response. Our data on the nodular basal cell carcinomas suggest that size may be a significant factor, since none of the largest nodular tumors showed complete regression. Stjernswärd and Levin (15) observed that the relationship between the intensity of the inflammatory reaction and the therapeutic response was inconsistent and unpredictable. Because of this inconsistency and because the degree of inflammation produced by DNCB was insufficient to produce scarring they suggested that tumor regression was not simply a "mechanical" result of a strong inflammatory reaction. We observed that some tumors treated with the primary irritant, croton oil, underwent complete regression. This suggests that an irritant inflammatory response, unrelated to the cell-mediated character of allergic contact dermatitis or to tumor-specific immunity, may be sufficient to destroy some basal cell carcinomas. Alternatively, croton oil may exert effects in addition to an irritant inflammatory response. The lack of effect of DNCB in a tolerant patient suggests that DNCB in the absence of an allergic contact dermatitis does not have a chemotherapeutic effect even at irritant concentration(s) and favors an immune mechanism. In this regard it is interesting to note that the patient with the best therapeutic response to DNCB was also the most sensitive on dilutional patch tests and showed a high degree of lymphocyte transformation to DNCB in vitro (13). Furthermore, a blastogenic factor(s) falling under the generic category of lymphokines, as defined by Dumonde et al. (6), was released by DNCB and isolated in the cell-free supernatant of peripheral blood leukocyte cultures from this patient (10). While the relationships of these in vitro events to events in vivo are currently poorly understood, they point in the direction of an immune mechanism. Discovery of the factors that determine or predict the therapeutic responses of individual tumors and patients may lead to a more effective use of DNCB.

Regardless of the mechanism of DNCB-induced regression of cutaneous neoplasms, from the practical therapeutic standpoint there is sufficient evidence to warrant further exploration of the current clinical usefulness of DNCB in those subjects with multiple basal cell carcinomas who would be considered for topical therapy. Both DNCB and topical 5-fluorouracil produce more vigorous reactions in tumor tissue than in adjacent normal skin and both induce inflammation and resolution of lesions that were subclinical prior to treatment (5, 8, 16). Both DNCB and 5-fluorouracil reportedly decrease the incidence of new lesions (8, 16), probably as a result of their ability to eradicate subclinical lesions. Whether "immunotherapy" with DNCB is superior to "chemotherapy" with 5-fluorouracil in decreasing the incidence of new lesions, a possibility suggested by Klein (8, 16), is unknown. In this study we observed no regression of untreated tumors indicating no "distant" effect of DNCB treatment, which is in accord with reported observations (15). The high failure rate of DNCB therapy for discrete tumors reported in this study currently precludes the use of DNCB as a primary mode of therapy for isolated basal cell carcinomas. DNCB may prove to be of greatest value in the treatment of numerous small lesions.

REFERENCES


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Fig. 1. Nodular (left) and superficial (right) basal cell carcinomas on the back of Patient 4 prior to therapy.

Fig. 2. Eleven weeks after one 8-week course of therapy, the superficial basal cell carcinoma has undergone complete clinical regression. The adjacent nodular lesion has shown no response.

Fig. 3. Nodular basal cell carcinoma of neck of Patient 5 prior to therapy.

Fig. 4. After one 8-week course of therapy, this lesion has undergone partial regression.

Fig. 5. Nodular basal cell carcinoma on back of Patient 5 prior to therapy.

Fig. 6. Three weeks after the 3rd 8-week course, this lesion has increased in size.

Fig. 7. Back of Patient 6 with basal cell nevus syndrome reveals confluent areas of basal cell carcinomas as well as some discrete lesions prior to therapy.

Fig. 8. Four weeks after one 8-week course of therapy, considerable clinical improvement has occurred. However, there is both clinical and histological evidence of persistent tumor.

Fig. 9. Detail of lower back illustrates area of confluent basal cell carcinomas prior to therapy.

Fig. 10. Detail of lower back after therapy illustrates the beneficial therapeutic response in this patient.
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