Delayed Hypersensitivity to 5-Fluorouracil following Topical Chemotherapy of Cutaneous Cancers

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SUMMARY

Skin tests to various common antigens, dinitrochlorobenzene, and 5-fluorouracil (5-FU) were performed on patients being treated for cutaneous neoplasms with topical 5-FU cream. Eleven of 15 patients tested both before and after therapy converted from skin test negative to positive with respect to 5-FU. This conversion correlated with positive dinitrochlorobenzene skin tests and therapeutic cure. The relation between the induction of delayed hypersensitivity reactions to 5-FU following treatment with topical 5-FU and the cure rate for cutaneous neoplasms showed a trend toward correlation.

INTRODUCTION

5-FU* has been used topically for several years in the treatment of malignant and premalignant skin lesions (7, 18, 22, 27–31). This form of treatment appears to be safe and effective and leaves little scarring. For premalignant lesions it is superior to other modes of treatment (42), and for the treatment of superficial basal cell carcinoma it is as effective as surgery or radiotherapy.

When applied topically 5-FU will penetrate active solar keratoses but will not penetrate normal skin (13), and at the site of penetration the incorporation of [3H]thymidine into DNA is prevented. If 5-FU is injected intradermally this metabolic block occurs in both normal and abnormal skin to an equal extent (13). Given systemically 5-FU has generally been regarded as immunosuppressive (41), even in very small doses (44). However, drug penetration of topically applied medication amounts to only 6% of the applied dose and does not cause immunosuppression (12).

5-FU incorporation into cellular RNA and its inhibition of DNA in the S phase of cell division appear to be the chief reasons for the anticancer action of the drug (9, 16, 34, 35). However, it may be that the incorporation of 5-FU into intracellular metabolites or organelles, possibly microsomes (36), may also be of importance.

MATERIALS AND METHODS

Patients studied were Caucasian adults being treated in the Tulane Topical Chemotherapy Clinic. All were suffering from basal, basosquamous, or squamous carcinoma or carcinoma in situ of the skin.

During treatment with topical 5-FU† patients were seen biweekly or triweekly and therapeutic results were recorded by serial photography. After completion of treatment each patient was followed for a minimum of 1 year. The method of treatment has been described fully elsewhere (29).

The immune status of 40 patients was evaluated, and all have been followed up to the present. In 25 patients (Group 1) previous treatment had been conducted here and elsewhere; sensitivities were determined from 2 weeks to 36 months after treatment.

In 15 other patients (Group 2) all sensitivities were determined before treatment. Skin tests with only 5-FU were then repeated from 2 weeks to 3 months after conclusion of topical chemotherapy. Pre- and posttreatment 1-mm punch biopsies were performed at treatment sites.

Delayed Hypersensitivity to the Following Skin Test Antigens. The following materials and techniques were used for sensitivity testing: PPD, 5 units/test dose (intermediate strength; Connaught Laboratories, Ltd., Willowdale, Toronto, Ontario, Canada); Dermatophytin (Mycobacterium scrofulaceum), 0.1 µg/test dose (Statens Seruminstitut, Copenhagen, Denmark); Dermatophyton (Trichophyton sp.), 1:30 solution in 0.9% NaCl solution; Hollister-Steir Labs, Spokane, Wash.) Dermatophyton O (Candida albicans). 1:100 solution in 0.9% NaCl solution;

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‡ Associate Professor of Surgery.
§ Professor of Pathology.
|| Professor of Surgery.
| The abbreviations used are: 5-FU, 5-fluorouracil; DNBC, dinitrochlorobenzene; PPD, purified protein derivative of tuberculoprotein.
| Received August 14, 1974; accepted February 7, 1975.

The clinical results seen in a number of patients treated with topical 5-FU suggested the possibility that cutaneous and generalized sensitivity to 5-FU developed in these patients, in much the same way as has been reported when Trenimon, ethyleneimino benzoquinone, DNBC, PPD, and nitrogen mustard are applied topically (17, 19-21, 26).

This research was undertaken to investigate the immune responses of patients with skin cancer before, during, and after treatment with topical 5-FU and also to determine whether topically applied 5-FU caused the development of delayed hypersensitivity to 5-FU.

1 Efudex cream, 5 and 20% concentrations, in an aquaphor cream base and Efudex lotion, 5% in propylene glycol; courtesy of Dr. Edward Miller, Director, Clinical Oncology Research, Hoffman-LaRoche, Inc., Nutley, N. J., and Dr. Edmund Klein, Chief of Dermatology, Roswell Park Memorial Institute, Buffalo, N. Y.
Hollister-Steir Labs, Spokane, Wash.); mumps skin test antigen (Eli Lilly & Co., Indianapolis, Ind.); and Varidase (1:100 dilution in 0.9% NaCl solution of streptokinase-streptodornase; Lederle Laboratories, Pearl River, N.Y.). These antigens were injected intradermally in 0.1-ml amounts on the anterior surface of 1 forearm, and the results were read after 48 hr. If induration of 4 mm or more was seen, the test was scored as positive.

Delayed Hypersensitivity to DNCB. Solutions were made up in dry acetone to give 2000, 100, 50, and 25 µg/0.1 ml. Fresh dilutions were made each week and stored at -20°. For sensitization a patch on the anterior surface of each wrist was cleaned with acetone and 0.1 ml of the appropriate solution was applied. DNCB (Matheson, Coleman & Bell, Norwood, Ohio), 2000 µg, was spread evenly on an area of 128 sq mm on 1 wrist and 100 µg DNCB was spread onto a similar area on the other wrist. Both areas were then covered with a Band-Aid for 48 hr. If a recall flare was seen 7 to 10 days later, the patient was challenged with 25 µg 14 days after sensitization. If no recall was seen, a challenge dose of 100 µg was used. Erythema and induration over the test area 48 hr after challenge was read as a positive response.

Delayed Hypersensitivity to 5-FU. Serial dilutions ranging between 5 and 0.04 mg/0.1 ml 5-FU (NDC-4-1904-6, Hoffman-LaRoche, Inc., Nutley, N. J.) in sterile, preservative-free water for injection were made up and given in 0.1-ml doses intradermally into the anterior surface of the forearm not used for the conventional skin test antigens. The results of these skin tests were read after 48 hr and scored as the others. In some cases 1-mm punch biopsies were performed on the test sites in order to study the histological features of the reaction.

Humoral Recognition Factor. The plasma levels of humoral recognition factors were measured by determining the influence of plasma on the uptake by the reticuloendothelial system of rat liver using a gelatinized 125I-labeled triolein lipid emulsion (37).

Serum Immunoglobulin Levels. Serum IgG and IgM levels were measured using standard commercially prepared assay plates (Meloy Laboratories, Springfield, Va.).

RESULTS

Of the 25 patients in Group 1 who had been previously treated with 5-FU (Table 1), 17 had positive skin tests to 5-FU and 8 had negative 5-FU skin tests. Of the 5-FU-positive subgroup, 13 (76%) had been cured of their disease and 4 (24%) had residual disease. Of the 5-FU-negative subgroup, 5 (63%) had been cured and 3 (38%) had residual disease. All of these 25 patients demonstrated a positive skin test to 1 or more of the other antigens used (Table 2).

The 15 patients in Group 2 were skin tested with 5-FU both before and after courses of treatment with topical 5-FU (Table 1). Eleven (73%) of these patients showed a conversion in their 5-FU skin test from negative to positive following treatment. One of these patients subsequently reconverted to negative 4 months after completion of therapy.

Of the 11 patients in Group 2 who developed a delayed hypersensitivity to 5-FU (Tables 2 and 3), 9 (82%) became DNCB positive. All of these patients were cured of their disease as demonstrated by gross examination. However, posttreatment biopsies revealed residual disease in 1 patient.

Of the remaining 4 patients in Group 2 who did not develop a positive skin test to 5-FU after topical chemotherapy (Tables 2 and 3), none became DNCB positive. Two patients in this subgroup were cured of their disease as evidenced by negative posttreatment examinations and biopsies. The remaining 2 patients showed persistent disease.

Only 1 of the patients in Group 2 was anergic to the conventional antigens. Gross and histological examinations of a posttreatment biopsy revealed, however, that he was also cured.

The plasma levels of humoral recognition factor activity were within normal levels for all the patients tested. The serum immunoglobulins, IgG and IgM, showed no significant deviation from normal values. Some were slightly elevated, but none were low and no consistent pattern emerged.

Pathological Examinations. A skin biopsy taken from a 5-FU test site (1.25 mg intradermally) at 48 hr (Fig. 1) showed an intact, atrophic epidermal surface with edema of the basal and suprabasal layers and mild lymphocytic and macrophage cellular infiltrates. The underlying papillary dermis and the full thickness of the reticular dermis showed an extensive round cell infiltrate filling the adventitial dermis and investing the vasculature. These vascular islands showed a preponderance of lymphocytes, but occasional macrophages as well as activated mesenchymal cells were present. The inflammatory cells spilled over into the adjoining dermal connective tissue, especially in the superficial reticular layer and portions of the papillary dermis. The latter also showed conspicuous edematous interstitial fluid accumulation. No evidence of epidermal necrosis, or vascular thrombosis, was present.

A DNCB (100 µg, challenge) test site biopsy (Fig. 2) showed changes identical to those in tissue from the 5-FU test site except for a lesser degree of papillary dermal edema. Vascular islands were characterized by well-estab-
Table 2

Skin test results in 40 patients treated with topical 5-FU

<table>
<thead>
<tr>
<th>Antigen</th>
<th>5-FU positive</th>
<th>5-FU negative</th>
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<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td></td>
<td>converted to</td>
<td>not converted</td>
</tr>
<tr>
<td></td>
<td>5-FU positive</td>
<td>to 5-FU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sensitivity</td>
</tr>
<tr>
<td><strong>DNCB</strong></td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td><strong>PPD</strong></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>Purified sensitin</strong></td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td><strong>R595</strong></td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td><strong>Mumps</strong></td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td><strong>Varidase</strong></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Trichophytin</strong></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Candida</strong></td>
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<td>5</td>
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</tbody>
</table>

* + , positive skin test to antigen listed; –, negative skin test to antigen listed.

Table 3

Detailed skin test results of Group 2 patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Converted to 5-FU positive in Patient</th>
<th>Not converted to 5-FU sensitivity in Patient</th>
</tr>
</thead>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>DNCB</strong></td>
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<td>+</td>
</tr>
<tr>
<td><strong>PPD</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Purified sensitin</strong></td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>R595</strong></td>
<td>+</td>
<td>+</td>
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<td><strong>Mumps</strong></td>
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<tr>
<td><strong>Varidase</strong></td>
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<td><strong>Trichophytin</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Candida</strong></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* + , positive skin test to antigen listed; –, negative skin test to antigen listed.

Discussion

In this study 11 (73%) of 15 patients who were skin tested with 5-FU intradermally both before and after treatment with topical 5-FU developed a delayed hypersensitivity to 5-FU. This phenomenon has not previously been described and may be a manifestation of the general immunological ability of patients with localized neoplastic disease to recognize new antigens as contrasted to the anergy often seen in advanced cancer (15).

The ability to become sensitized to 5-FU also corresponded closely with the ability to respond to DNCB and with therapeutic cure. Of the 4 patients in Group 2 who did not develop 5-FU hypersensitivity, none was able to develop a DNCB sensitivity and 2 patients (50%) had residual disease after treatment. However, of the remaining 11 patients in Group 2 who did develop 5-FU hypersensitivity, 9 (82%) developed DNCB hypersensitivity; of the other 2 patients who did not develop DNCB hypersensitivity, only 1 (9%) was not completely cured of his disease. These data suggest that the tumoricidal effect on skin cancers of topically applied 5-FU may be related not only to its antimetabolite action but also to an immunological action.

In some of the patients herein reported, previously unrecognized foci of disease adjacent to the area being treated often became inflamed during the course of treatment with 5-FU. This phenomenon has been recognized previously and has been of use in definition of disease-free areas to be used as skin grafts (40). It may also be an expression of an immunological response elicited against malignant tissue that has been altered as a result of the local infiltration of round cell infiltrates. The deep dermis was not represented in the biopsy.

A biopsy of a PPD (5 units, intermediate strength) test site showed a similar histological pattern to the 5-FU and DNCB test biopsies. These appearances are typical of those described for the delayed hypersensitivity responses to tuberculin (2) and other substances.

A 5% 5-FU cream treatment site was also biopsied 14 days after the beginning of therapy (Fig. 3). This showed a surface of organizing fibrinous exudate detached from the ulcerated surface. No epithelium or residual basal cell carcinoma was present, and there was a heavy band-like infiltrate of round cells involving the dermal supporting layer extending to the midreticular dermis level. The connective tissue in this band-like zone had been replaced by granulation tissue characterized by reactive proliferation of capillaries and intense lymphoid inflammatory infiltrate extending to a depth of 0.6 mm. Smaller numbers of plasma cells, macrophages, and proliferating fibroblasts were present. These latter cellular constituents are more conspicuous than were noted in the DNCB, PPD, and 5-FU test sites. A biopsy of a PPD (5 units, intermediate strength) test site showed a similar histological pattern to the 5-FU and DNCB test biopsies. These appearances are typical of those described for the delayed hypersensitivity responses to tuberculin (2) and other substances.

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use of 5-FU. The action of systemic 5-FU as an antimetabolite has been studied (9, 16, 34–36). However, the mechanism of induction of an immune response following topical 5-FU administration is not known. It is possible that this response could be the immunological recognition of cellular constituents altered by the incorporation of 5-FU into cellular RNA by substitution in place of normal uracil. This 5-FU complex, rather than being simply cytotoxic as a result of metabolic competition, might also act as an immunogen.

Klein et al. (22) noted that concurrent administration of topical 5-FU and DNCB caused antitumor effects in skin cancers that were considerably more intense than when either was used separately (21). In other research, Litwin et al. (30) described the possible immunological action of topical 5-FU when used to treat basal and squamous skin cancers. In this research comparison of the histology of the 5-FU and DNCB test sites with the 5% 5-FU treatment site of a basal cell carcinoma showed striking similarities in the cellular reaction. This combination of reactive cellular constituents was interpreted as being consistent with the cellular mechanism for delayed hypersensitivity (24, 25, 45).

The DNCB and 5-FU test sites showed characteristically dense vascular islands situated in the reticular and papillary dermis. These islands might well represent the distribution of antigen. The dense band-like infiltrate characterizing the posttreatment site might also reflect antigen distribution. Its presence, almost exclusively in granulation tissue emerging after 5-FU therapy, corresponded with the location of the cancer and with a fairly uniform absorption pattern of 5-FU. By location and constituency, it was also consistent with a delayed hypersensitivity reaction that destroys tumor.

An alternative explanation for the histological pattern of the treated lesion could be that it merely represented the local effect of chemical irritation caused by 5-FU. However, if this were the case, the histological picture would have been one of tissue necrosis, edema, fibrinoid vascular change, and marked neutrophil response, as is seen with the use of caustic compounds (5) and in burn injuries (37). Furthermore, the degree of round cell response seen in a pure chemical injury, following the acute phase, is limited. It appears more likely that the histological picture seen was that of a typical delayed hypersensitivity reaction (24, 25, 45).

Tumor cell membrane is antigenically different from that of normal cells, and it is likely that tumor cell membrane precursors also differ from their normal counterparts. 5-FU is capable of forming complexes with RNA (34). Thus it is possible that altered membrane, membrane precursors, or membrane surface components may be capable of eliciting an immune response in lesions treated with topical 5-FU where none was evident before.

It is also possible that small, slowly growing malignant lesions could induce a state akin to immunological tolerance, thus “sneaking through” immunological defenses, as has been described in animal experiments (23). If this were so, then the breaking of this immunological tolerance might be brought about by the addition of chemical groups to the tolerogen, a well-known experimental maneuver (46). If this could occur, then the patient’s immune defenses might be capable of recognizing the malignant cell altered by its added marker, and thus of rejecting the cancer. This phenomenon could explain both the clinical and histological pictures seen in patients treated successfully with 5-FU.

A similar mechanism has been suggested to explain the observation that malignant cells coupled with foreign proteins (10, 11, 47), chemicals (3, 39), plant substances (14), and cytotoxic agents (1) elicit an immune response which the malignant cells by themselves were unable to do (15). Microsomal-DNCB complexes have been shown to act as immunogenic antigens in the induction of contact sensitivity to DNCB (36). Further, Miller and Levis (33) have reported the transformation of lymphocytes during the course of DNCB sensitization, thus demonstrating that chemical agents can cause measurable immunological responses when combined with cellular protein. Intradermal injections of DNCB have also been shown to produce increased immunological responsiveness (8).

Other mechanisms of hypersensitivity induction must also be considered. One alternative explanation might be the formation of fraudulent RNA, compatible with cellular survival for long enough periods to produce fraudulent proteins or other macromolecules that then may act as immunogens. The complexing of 5-FU with a surface component of normal neoplastic cells, thus producing immunogens, is another possibility. Furthermore, 5-FU may be acting as a hapten rather than as a complete immunogen.

In the patients studied in this research the delayed hypersensitivity responses to conventional skin test antigens, plasma humoral recognition factor activity, and serum immunoglobulin levels were not depressed. This is in contrast to the depressions that are associated with disseminated cancer (32, 38). This is almost certainly due to the fact that the patients in this series presented with a minimal tumor load, were in good general health, and were treated only with very small doses of chemotherapeutic agents applied topically, rather than administered systemically. In other research in these laboratories no delayed hypersensitivity to 5-FU has been seen in patients with breast and colon cancers being treated with 500 mg 5-FU i.v. at weekly intervals.

In contrast to the generally held assumption that 5-FU is immunosuppressive (6, 41, 44), this study indicates that this drug, under the special circumstances of this research, may act as an immunological stimulant. It appears that a delayed hypersensitivity challenge reaction to 5-FU or a reaction resembling it was elicited in a significant number of patients receiving topical 5-FU. The relation between the induction of delayed hypersensitivity challenge reactions following topical 5-FU and the cure rate for cutaneous neoplasms showed a trend toward correlation. The immunological status of further numbers of such patients must be studied before these theories can be verified, but similar results have also been reported in patients treated with a variety of cytotoxic agents in whom increased, rather than decreased, immunological responsiveness resulted after chemotherapy (4, 8, 43). This application of certain chemotherapeutic agents, particularly 5-FU,
as immunogens may be of important consequence in the development of chemoimmunotherapeutic techniques.

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Delayed Hypersensitivity to 5-FU


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Fig. 1. A, biopsy of 5-FU skin test site (1.25 mg/0.1 ml intradermally). Well-developed vascular islands are present in the papillary and reticular dermis. In the superficial layer, there is spillage of exudate into the neighboring connective tissue. The overall picture is typical for a delayed sensitivity reaction. H & E, × 64. B, biopsy of 5-FU skin test site (enlarged). The perivascular round cell infiltrate is composed principally of lymphocytes, extends into vascular mural structures, and is accompanied by active fibroblasts in the adjoining dermis. H & E, × 640.

Fig. 2. A, biopsy of DNCB skin test site (100 µg/0.1 ml). The morphological picture is identical with that seen in Fig. 1 except for a moderate tangential section artifact. The vascular islands are also well developed, and the epidermis remains intact. H & E, × 64. B, biopsy of DNCB skin test site (enlarged). Lymphocytes also constitute the major cell constituent in this dense infiltrate. H & E, × 640.

Fig. 3. A, biopsy of basal carcinoma being treated topically with 5% 5-FU cream. The surface is denuded with a small amount of detached fibrinous exudate. A dense band-like infiltrate of round cells is present in the superficial dermis. The adjoining middermis is moderately involved while the deep dermis shows no exudate. No residual cancer is present. H & E, × 64. B, biopsy of basal carcinoma being treated topically with 5% 5-FU cream (enlarged). The exudate contains a conspicuous lymphocyte content and an increased number of macrophages is present. Emigration of neutrophils in the dilated capillary to the right is noted. The overall picture is interpreted as a delayed sensitivity reaction also exhibiting a cellular response attributable to surface denudation. H & E, × 640.
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