Trimethylpsoralen Bath PUVA Is a Remittive Treatment for Psoriasis Vulgaris

Evidence That Epidermal Immunocytes Are Direct Therapeutic Targets

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Background: Psoriasis vulgaris can be effectively treated with trimethylpsoralen (TMP) bath PUVA therapy (psoralen plus UVA), but no data exist on the extent to which psoriatic pathology is affected by this treatment, or on its cellular mechanism of action.

Observations: Eleven patients with recalcitrant psoriasis vulgaris were treated with TMP bath PUVA therapy and observed through clinical and histological measures. Clinical resolution of psoriasis was achieved in 10 of 11 patients. Histopathological resolution of epidermal hyperplasia (marked by keratin 16 expression) was achieved in 90% of individuals treated with TMP bath PUVA. Epidermal acanthosis was reduced by 40% at 2 weeks and 66% by the end of treatment. Epidermal improvement correlated best with reduction in intraepidermal T lymphocytes, which were reduced by 76% at 2 weeks of treatment and 93% at the end of treatment. Furthermore, following TMP bath PUVA therapy, the numbers of epidermal CD1a+ Langerhans cells were markedly reduced, and CD86+ cells were eliminated. Through in vitro assays, TMP was found to be about 10 000-fold more active as a lymphotoxic agent compared with 8-methoxypsoralen (8-MOP). Additionally, at physiologic concentrations, lymphocytes were killed more readily by TMP PUVA (TMP plus UVA) than were keratinocytes.

Conclusions: Treatment with TMP bath PUVA was effective in treating moderate to severe psoriasis, even in darker pigmented individuals. It is likely that this treatment ameliorates psoriasis through direct effects on activated leukocytes in lesional skin.

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CONVENTIONAL PUVA therapy (oral administration of 8-methoxypsoralen [8-MOP] followed by UV-A irradiation) has been used successfully for more than 20 years to treat difficult cases of psoriasis. While short-term adverse effects such as nausea and malaise limit treatment in some patients, there is increasing concern over PUVA-induced cutaneous carcinomas and melanomas that will effectively limit its long-term use in most patients. Bath PUVA therapy has been suggested as a potentially safer alternative to conventional

RESULTS

CLINICAL OUTCOME MEASURES

The Table summarizes the clinical responses to TMP bath PUVA therapy in our patient population. These patients had skin types ranging from Fitzpatrick types II to VI, but more than 50% of patients who completed treatment had skin types V or VI. All patients had significant improvement in clinical severity measures. Two weeks after starting treatment, a 25% mean improvement in plaque severity was measured (P<.001). At the end of treatment, an 83% mean reduction in disease severity was measured (P<.001). All patients showed clinical benefit, with 10 of 11 patients having clear skin or only trace dis-
PATIENTS AND METHODS

PATIENTS

Thirteen patients (11 men and 2 women) with long-standing psoriasis vulgaris (mean disease duration, 16 years) were sequentially enrolled into our TMP bath PUVA study. These patients had 10% to 90% (mean, 44%) skin surface involvement and had been treated previously with a variety of agents. Six patients had Fitzpatrick skin types V or VI. Eleven of 13 patients completed the study, while 2 patients were noncompliant with treatment and were dropped from the study. Data from 1 of the 11 patients who completed the study (who achieved clinical clearing) were not included in histological analyses because the final biopsy was refused.

TREATMENT

Minimum phototoxic dose (MPD) testing to UVA was performed by soaking an extremity (usually an arm) in a TMP solution of 0.167 mg/L for 15 minutes and then exposing 2 × 2-cm patches to UVA in amounts ranging from 0.2 to 2.0 J/cm². Photopatches were read at 12, 24, 48, and 72 hours following UVA exposure. The starting UVA dose was 30% to 75% of the MPD. In patients for whom the MPD was indeterminate, conservative initial exposures of 0.2 to 0.4 J/cm² were given. Treatment was delivered on an alternate-day schedule. Subsequent doses were increased by 0.1 to 0.5 J/cm², depending on skin type and lack of phototoxic effects from prior treatment. The goal for each treatment was to attain slight (+/-) erythema following UVA exposure. The patients were supplied with an emollient (Aquabase) to apply to their skin as desired.

The TMP was purchased as 5-mg tablets for oral administration (ICN Pharmaceuticals, Costa Mesa, Calif). An ethanol solution was prepared by crushing tablets and extracting the powder, then combining it with 20 mL of absolute ethanol at 60°C for 12 to 24 hours, after which the solution was filtered to remove suspended filler. A measured amount of TMP solution (25 mg in 100 mL of ethanol) was added to 150 L of lukewarm water in a bathtub (final concentration, 0.167 mg/L). The patient then sat submerged up to the neck for 15 minutes. Following the bath, the patient dried off with a towel and immediately entered the light box while wearing UV-protecting glasses and a groin shield. The phototherapy unit (model 57000, Psoralite Corporation, Columbia, SC) delivered approximately 13 mJ/cm² of UVA (measured with a UVA meter from National Biological Corporation, Twinsburg, Ohio).

HISTOLOGICAL ANALYSIS

Biopsies of lesional skin were performed before treatment, after 2 weeks of treatment, and at the end of treatment. Skin biopsy specimens were frozen in optimum cutting temperature compound solution for histological analysis. Cryostat sections were stained with CD3 (Becton Dickinson, San Jose, Calif), Ks8.12 (Sigma Aldrich Inc, St Louis, Mo), CD1a (Becton Dickinson), or CD86 (FUN-1 clone, Pharmingen, San Diego, Calif) monoclonal antibodies as previously described.15 Epidermal thickness was measured on digitized micrographs using the National Institutes of Health Image software.16

Reduction in Disease-Related Parameters During Treatment*  

<table>
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<th>Patient No.</th>
<th>PSI (2-wk) Parameters</th>
<th>End of Treatment Parameters</th>
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<tr>
<td></td>
<td>Epidermal Thickness</td>
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<tr>
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</tr>
<tr>
<td>Mean</td>
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*PSI indicates scores on the Psoriasis Severity Index. All data are presented as percentage of reduction.

Biopsy specimens obtained from lesional skin before treatment, after 2 weeks, and at the end of treatment were examined for disease-related pathology using histochemistry and computer-assisted image analysis. As shown in the Table, mean epidermal thickness was reduced by 40% after 2 weeks (P<.001) and 66% by the end of treatment (P<.001). To determine whether pathological keratin expression was reversed by this treatment, biopsy specimens were examined for the expression of keratin 16 (Figure 1). Keratin 16 was expressed in suprabasal ke-
ratinocytes in all pretreatment biopsy specimens and continued to be expressed in 9 of 10 patients after 2 weeks of PUVA treatment. At the conclusion of treatment, keratin 16 was expressed in only 1 of the 10 psoriatic lesions. Because keratin 16 is expressed only in hyperplastic epidermis undergoing “regenerative” maturation,17 these staining results indicate reversal of regenerative growth by TMP bath PUVA treatment.

Previous studies have suggested that T lymphocytes may be the major cellular target of PUVA (8-MOP plus UVA) therapy.18,19 Accordingly, we sought to determine the lymphocyte-depleting effects of TMP PUVA therapy in psoriatic tissue and to measure direct lymphotoxic effects of TMP in vitro. The effects of TMP bath PUVA therapy on CD3+ lymphocytes in psoriatic lesions are presented in the Table. Two weeks after starting PUVA treatment, there was a striking reduction in intraepidermal T lymphocytes (mean decrease, 76%; P<.001), but only a modest reduction (mean decrease, 22%) in T lymphocytes located in the papillary dermis. By the end of treatment, intraepidermal T lymphocytes had been reduced by 93% and dermal lymphocytes had been reduced by 62%. After 2 weeks of treatment, reductions in epidermal acanthosis were highly correlated with reductions in intraepidermal CD3+ cells (r = 0.87), and less correlated with dermal T-lymphocyte reductions (r = 0.58). For all study points (Figure 1) epidermal thickness was more highly correlated with the number of epidermal T lymphocytes compared with dermal cells. These data are consistent with

Figure 1. Keratin 16 expression in psoriatic lesional epidermis before (A), at 2 weeks (B), and after trimethylpsoralen (TMP) therapy (C). The effects of TMP bath plus UVA therapy on epidermal and dermal T-lymphocyte infiltration as visualized by CD3+ antibody staining on skin biopsy specimens obtained from another patient before (D), at 2 weeks (E), and after treatment (F).
epidermal changes being induced primarily by the intraepidermal T-lymphocyte subset.

The ability of PUVA to decrease inflammation in psoriatic lesions could also be mediated through its depleting actions on Langerhans cells.20-24 In the unaffected skin of patients with psoriasis, CD1a+ Langerhans cells are present throughout the epidermis, but few cells express detectable levels of the costimulatory molecule B7-2 (CD86), as shown in Figure 2. In contrast, CD1a+ cells are mostly located in more differentiated regions of lesional epidermis and numerous CD86+ cells are also present in these areas. Following TMP bath PUVA therapy, there was a marked reduction in the number of CD1a+ cells in the epidermis, and CD86+ cells were eliminated. Hence, Langerhans cells (particularly activated CD86+ cells) appear to be depleted from psoriatic lesions by TMP bath PUVA treatment.

**IN VITRO STUDIES**

Finally, we sought to determine the relative cytotoxic effects of TMP and 8-MOP on activated lymphocytes. Previous studies indicated that 8-MOP plus UVA can selectively induce T lymphocytes to undergo apoptotic cell death.18,19 We used several different assays to evaluate the effects of TMP on mitogen-activated peripheral blood T lymphocytes. Since apoptotic cells eventually disintegrate into subcellular apoptotic fragments, PUVA-induced cytotoxic effects can be quantified by measuring reductions in a particular target cell population. Hence, the cytotoxicity

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*Figure 2. The effects of trimethylpsoralen (TMP) bath plus UVA therapy on expression of CD1a and CD86 in dendritic cells in normal skin (A and B), lesional plaques (C and D), and treated lesional skin (E and F). Arrows indicate dendritic CD86+ Langerhans cell, with close-up in lower right corner (D). Black bars indicate 100 µm.*
of 8-MOP or TMP for T lymphocytes was assessed by enumerating CD3+ cells through flow cytometry. T lymphocyte numbers were reduced 89% by treatment with 0.01 ng/mL of TMP PUVA, 98% by treatment with 0.1 ng/mL of TMP PUVA, and 92% by treatment with 100 ng/mL of 8-MOP plus UVA. Cultures showed T-lymphocyte size and granularity shifts that typify apoptosis19 and subgenomic DNA fragmentation using flow cytometry.18 Based on relative concentrations of psoralens required to produce cytotoxicity, mitogen-activated T lymphocytes appeared to be about 10,000-fold more sensitive to TMP than to 8-MOP.

Finally, the relative sensitivity of epidermal keratinocytes vs lymphocytes to TMP PUVA therapy was measured using flow cytometry–based viability and cell counting assays. As was the case for 8-MOP PUVA therapy,18 lymphocytes were killed by TMP PUVA therapy, but keratinocytes were relatively resistant (data not shown).

Currently, psoriasis is considered to be an immune-mediated disease in which activated T lymphocytes trigger rapid keratinocyte proliferation, altered epidermal differentiation, and neovascularization.25 In turn, the activation of T lymphocytes is regulated by dendritic cells in psoriatic lesions that have up-regulated B7 costimulatory molecules and can serve as potent stimulators of resting T lymphocytes.26 The clinical importance of an immune pathoetiology is that it may be possible to develop immune-directed therapy that minimizes toxicity to other cells. In fact, 8-MOP is a potent cytotoxic agent for T lymphocytes following activation by UVA, but it is not entirely selective in its actions.18,19 Previous studies have shown that 8-MOP, when used in PUVA bath treatment, produces profound depletion of lesion-infiltrating T lymphocytes and that pathological epidermal hyperplasia is reversed following a course of treatment.15 Although both TMP and 8-MOP PUVA therapy reverse pathologic keratinocyte hyperplasia (defined by keratin 16 expression), one important difference is that epidermal Langerhans cells were strongly depleted by TMP PUVA therapy in lesional psoriatic epidermis, whereas 8-MOP did not produce marked reductions in lesional Langerhans cells.15 The ability of TMP PUVA therapy to reduce expression of CD86 in epidermal cells (presumably Langerhans cells) provides yet another mechanism for immune suppression, ie, T-cell co-stimulation should be diminished due to less B7 expression on dendritic cells.

Clinical observations suggest that TMP bath PUVA therapy might be less carcinogenic than conventional PUVA.10-14 In animal and bacterial model systems, TMP and 8-MOP have been found to be potent carcinogens.27-31 It is possible that TMP will be proven to be highly carcinogenic in psoriatic patients once sufficient exposure is given, and this possibility cannot be excluded with certainty by present data.10-14 However, if this type of treatment does eventually prove to be less carcinogenic, one potential explanation could be that topical psoralens cause different cellular effects than systemic psoralens. Based on previous studies,16 it is likely that intraepidermal T lymphocytes (as well as Langerhans cells that support ongoing T-lymphocyte activation) are the main cellular targets for therapeutic improvement. Epidermal psoralen levels have been measured at higher than 250 pg/g from bathing in TMP solutions equivalent to those used in this study.35 Since TMP concentrations of only 10 pg/mL were highly cytotoxic for activated T lymphocytes in vitro studies, actual psoralen levels attained in the epidermis from in vivo treatment should be sufficient to target T lymphocytes (even if UVA levels that penetrate epidermis are <2 J/cm2). The marked depletion of intraepidermal T lymphocytes following TMP bath PUVA therapy is consistent with the direct cytotoxic effects on this cell type, but the reduced T-lymphocyte infiltration could also be mediated by elimination of CD86+ dendritic cells from skin lesions. Conceivably, topically applied TMP might have limited penetration in the epidermis such that cross-linking of DNA occurs mostly in differentiated cell layers (where CD3+ and CD86+ cells predominate). It is theoretically possible that therapeutic effects could be separable from carcinogenic effects based on differential psoralen distribution and differences in the location of target cells.

In the end, we found TMP bath PUVA therapy to be well tolerated and highly effective at producing clinical and histological resolution of psoriasis. The ability of TMP bath PUVA therapy to eliminate activated Langerhans cells and T lymphocytes within the epidermis is likely to underlie its potent therapeutic actions in psoriasis. Although safety data on the carcinogenic risk of TMP bath treatment are less complete than for oral 8-MOP, we believe that TMP bath PUVA therapy represents a sensible alternative for the present.

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