Comparison of the Effect of 8-Methoxypsoralen (8-MOP) plus UVA (PUVA) on Human Melanocytes in Vitiligo Vulgaris and In Vitro

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In this study, we examined the various effects of PUVA treatment on cultured human melanocytes, and it revealed that 1) the higher the dose of PUVA treatment, the more significant the inhibition of cell DNA and protein synthesis; 2) the higher the dose of PUVA treatment, the more significant the depletion of epidermal growth factor receptor (EGFR) expression; 3) PUVA treatment at 124 mJoule/cm² depleted the vitiligo-associated melanocyte antigens (VAMA) immediately after irradiation, and both the VAMA and EGFR expression progressively recovered at 24 or 72 h after PUVA; 4) PUVA treatment stimulated tyrosinase activity, but not in a dose-dependent fashion. In vitiligo vulgaris, PUVA treatment may stimulate the regrowth of melanocytes from hair follicles, but deplete the epidermal Langerhans cells in depigmented lesion of patients with stable vitiligo.

Comparing the above results obtained from in vivo and in vitro studies, it reveals significantly different biologic responses. Although the precise therapeutic mechanism of PUVA treatment in vitiligo is still not well known, it is proposed that 1) PUVA treatment may stimulate the other components of skin, such as keratinocytes, to release inflammatory mediators and some of them may act as melanocyte growth-stimulatory factors (MGSF), which further enhance the proliferation of remaining melanocytes in hair follicle; and 2) PUVA treatment may deplete the VAMA expression on cell membrane of melanocytes and also deplete epidermal Langerhans cells, which may result in blocking the progressing of antibody-dependent cell-mediated cytotoxicity to melanocytes in vitiligo. J Invest Dermatol 98:734–740, 1992

The psoralens, when activated by ultraviolet light A (UVA), are potent modulators of epidermal cell growth and differentiation. Clinically, the combination of psoralen and UVA (PUVA) as a model of phototherapy has been used in a wide variety of cutaneous disorders such as psoriasis and vitiligo vulgaris [1], and a great deal of effort has been expanded to elucidate the mechanisms by which PUVA alters normal cellular function [2]. The photoreaction of PUVA on DNA (type I reaction) is well studied. On absorption of photons, the psoralen molecule may either form monooxids with DNA or may become crosslinks [3][4]. Various irradiation schedules have been applied in culture systems to look at the effects of monooxids, crosslinks, and combinations of both on cell killing, mutation, and repair [5–7]. The PUVA-induced inflammatory reaction is most likely the result of a type II reaction. It may be that the dissipation of energy disrupts organelles containing inflammatory mediators or inducing their synthesis after a temporary period of metabolic inactivation. Immunologically, PUVA treatment leads to impairment of epidermal Langerhans cell function, and alternation of circulating T- and B-cell function [8][9]. PUVA treatment results in numerical and morphologic changes in the melanocyte system of the C57BL mouse, and UVA irradiation alone has no significant effect on the melanocyte number [10]. Inflammation and destruction of the epidermis caused by the PUVA treatment is followed by regeneration of the epidermis and melanocyte system. Although the effects of PUVA on keratinocytes, fibroblast, lymphocytes, melanoma cells, and melanocytes of mice have been well studied, there are still few reports concerning the effects of PUVA on melanocytes in patients with vitiligo vulgaris and in vitro. In vitiligo vulgaris, it has been proposed that all three kinds of epidermal cells, including Langerhans cells, keratinocytes, and melanocytes, are involved in the pathogenesis [11][12]. In this study, we examine the effects of PUVA on cultured human melanocytes, including vitiligo-associated melanocyte antigens, epidermal growth-factor receptor, tyrosinase activity, and DNA and protein synthesis. Furthermore, we also compare the dynamic change of epidermal melanocytes and Langerhans cells in patients with stable vitiligo vulgaris before and after a course of PUVA treatment. Finally, we
propose a possible therapeutic mechanism of PUVA photochemotherapy in vitiligo vulgaris according to in vivo and in vitro studies.

MATERIALS AND METHODS

Cell Culture The preparation of skin for melanocyte culture was performed in a manner as reported by us previously [13]. In short, the foreskins of adults from routine circumcision were cleaned of excess subcutaneous tissue, cut into small pieces (5 x 5 mm), and incubated with calcium- and magnesium-free phosphate-buffered saline (PBS, pH 7.2) containing 0.25% trypsin (Gibco, Santa Clara, CA) at 4°C overnight. The epidermal sheets were separated from dermis by fine forceps, and then the epidermal cell suspension was prepared as reported before [14]. The cells were pelleted by centrifugation (500 x g, 10 min) and resuspended in serum-free medium (SFM) containing MCDB 151 medium (Gibco) supplemented with epidermal growth factor (EGF, 10 ng/ml), hydrocortisone (HC, 10^-7 M), insulin (1, 5 μg/ml), choler toxin (CT, 2 ng/ml), transferrin (T, 10 μg/ml), bovine pituitary extract (BPE, 30 μg/ml), isobutylmethyl xanthine (IBMX, 10^-4 M), penicillin (100 U/ml), and streptomycin (100 μg/ml). The CT and IBMX were purchased from Sigma (St. Louis, MO) and the EGF, T, HC, I, and BPE were purchased from Collaborative Research, Bedford, MA. The epidermal cell suspension was plated onto plastic 25 cm flasks (Falcon Plastics 3013, Oxnard, CA) at a seeding density of 5 x 10^6 cells per dish, and incubated at 37°C in a humidified atmosphere containing 10% CO2. Twenty-four hours after primary seeding, the medium was changed, and then changed regularly every 2 days.

About 7 to 10 days after primary seeding, a pure human melanocytes culture was obtained, which was identified by dopa reaction and electron microscopic examination [15]. The semiconfluent purified human melanocytes were incubated with 0.25% trypsin + 0.01% EDTA solution (Gibco) at 37°C for 3-5 min, harvested with fetal bovine serum (FBS, Gibco), and centrifuged (500 x g, 10 min), and the cell pellet was re-suspended in SFM and re-inoculated at a density of 1 x 10^5 cells per flask. The fourth-passaged cells, about 25-35 days in culture, were used as materials for evaluating the effects of PUVA on melanocytes.

Experimental Procedures To assay the effect of PUVA treatment on EGF expression, tyrosinase activity, protein, and DNA synthesis of melanocytes in vitro, the cells were seeded onto 35-mm dishes at a density of 2 x 10^6 cells per dish. Twenty-four hours after seeding, the cells were incubated with 8-methoxypsoralen (8-MOP, Sigma, 2 x 10^-4 M in SFM) at 37°C for 30 min in the dark. Then the dishes were washed with 2 ml SFM three times; the medium was aspirated; the covers of dishes were opened; and cells were exposed to various dose of UVA irradiation, including, 0, 30, 60, or 124 ml/cm². The UVA irradiation was obtained by using a VL-15L 365-nm tube (S L Viller Lourmat, Marnela Vallee, France), and the dose of irradiation was measured by a UVU radiometer (UVP, San Gabriel, CA).

Cell Viability Twenty-four hours after PUVA treatment, the cells in dishes were stained with ethidium bromide (EB, Sigma, 0.0004% in PBS) at room temperature for 5 min [16] and a differential count of 200 cells was observed by using an Olympus IMT-2 inverted fluorescence microscope. In damaged cells, the EB entered into nuclei and intercalated with DNA to emit orange-red fluorescence.

Epidermal Growth-Factor Receptor (EGF) Assay Immediately, 24 h, and 72 h after various doses of PUVA treatment, the cells were incubated with 0.25% trypsin + 0.01% EDTA solution (Gibco) for deattachment; harvested by centrifugation; fixed with 100% cold methanol (Sigma) at -20°C for 3 min; incubated with mouse anti-epidermal growth-factor receptor antibody (BioMarker, Kiryat Weizmann, Israel, 1:20 diluted with PBS) at 37°C for 30 min; and incubated with FITC-conjugated goat anti-mouse immunoglobulin (BioMarker, 1:20 diluted in PBS) at 37°C for 30 min. Between each step, the cells were washed with PBS by centrifugation. The cells were adjusted to 2 x 10^5 cells per ml in PBS for flow-cytometric analysis. The cells were analyzed on FACScan Consort 30 (Beckton Dickinson, Sunnyvale, CA) using 488-nm excitation light [17]. In order to define autofluorescence, the cells were incubated with FITC-conjugated goat anti-mouse immunoglobulin only, and autofluorescence of cells was designated as background.

DNA and Protein Synthesis Assay Immediately after PUVA irradiation, the medium of all control and experimental groups was replaced with 2 ml SFM containing 5 μCi/ml [methyl-3H] thymidine (specific activity 25 μCi/mm, from Amershams International, Bucks, UK) for DNA synthesis assay, or with 2 ml SFM containing 2 μCi/ml L-(U-3C) proline (specific activity 260 mCi/mm, from Commissariat a l'Energie a l'Tomique) for protein synthesis assay. After incubation for 24 h, the cells were washed three times with PBS, resuspended by incubating with trypsin-EDTA solution to make a cell suspension, harvested, and washed by centrifugation (500 x g, 10 min). The cell pellets were lysed in 1 N NaOH, and incorporated tritiated 3H-thymidine and 3C-proline was counted after adding hydrofluor (National Diagnostics, Manville, NJ). The radioactivity was counted in a liquid scintillation counter (Minaxi-B Tri-Carb 4000 Series). The amount of 3H-thymidine incorporated was expressed as counts per minute, and as percentage of control.

Tyrosinase Activity Assay Immediately after PUVA irradiation, the medium was replaced with 2 ml SFM containing 2 μCi/ml 3H-tyrosine (NEN Products, Boston, MA) for each dish including control and experimental groups. After incubation for another 24 h, the medium was collected for tyrosinase assay. The amount of H2O released into the medium when 3H-tyrosine was converted by tyrosinase into 3,4-dihydroxyphenylalanine was measured and performed in a manner as described previously [13], and counted by a liquid scintillation counter. Tyrosinase activity was expressed as counts per minute, and as percentage of control.

Figure 1. EGF flow-cytometry analysis. Nonspecific autofluorescence background was defined as that to the left of the vertical boundary line (A). Immediately after PUVA, the percentage of the total cells counted showing specific FITC-EGF conjugated fluorescence was 1) in the control group B, 9.4 ± 0.7%; 2) in PUVA 31 μM/cm² (C), 6.9 ± 0.6%; 3) in PUVA 62 μM/cm² (D), 3.3 ± 0.3%; and 4) in PUVA 124 μM/cm² (E), 0.3 ± 0.1%.
Effect of PUVA on Vitiligo-Associated Melanocyte Antigens (VAMA)

**Serum:** The sera of 19 patients with active non-segmental—type vitiligo who had new depigmented lesions within 3 months [18] and 12 normal controls were collected for indirect immunofluorescence examination.

**Cells:** Twenty-four hours after plating onto glass coverslips in 35-mm culture dishes, the cells were incubated with 2 × 10^7 M 8-MOP at 37°C for 30 min in the dark; the medium was aspirated to leave 0.2 ml SFM per dish; the covers of dishes were opened and cells were exposed to 124 mj/cm² UVA irradiation. The post-irradiated cells were immediately subjected to indirect immunofluorescence test (IIF), or incubated for another 24 h in the dark and then subjected to IIF examination.

**Indirect Immunofluorescence Test:** First, the cells plated on coverslips were fixed with 100% methanol at −20°C for 3 min; incubated with serum (1:5 diluted in PBS) at room temperature for 60 min; and incubated with FITC-conjugated rabbit anti-human IgG (DAKO: 1:50 diluted in PBS) at room temperature for 60 min. Between each step, the cells were washed with PBS three times. The samples were observed by using an Olympus IMT-2 inverted fluorescence microscope.

Enzyme-Linked Immunosororbent Assay (ELISA) In order to detect the VAMA, a sensitive cellular ELISA was used. The assay procedures were modified from the method described by Hashemi et al [19]. The melanocytes were subcultured in a 96-well flat-bottom tissue culture plate (Falcon 3072, Becton Dickinson, Oxnard, CA) at a concentration of 2 × 10⁴ cells per well. After 24-h culture, the medium was replaced with 0.15 ml SFM containing 2 × 10⁻⁷ M 8-MOP in each well and incubated for 30 min; then, after being washed with SFM, the cells were exposed to either 124 mj/cm² UVA or no irradiation. The cells were taken for ELISA immediately, 24 or 72 h after PUVA. Then the cells were fixed in ice-cold 100% methanol for 3 min and, after washing three times with PBS, 150 µl diluted sera (1:4 diluted in PBS) was added to each well and the cells were incubated at room temperature for 1 hour. After washing three times with PBS, 100 µl of 1:50 diluted rabbit anti-human IgG (Cappel, Organon Teknika Coorporation, West Chester) was added to each well and the cells were incubated for 1 h at room temperature. The unbound conjugate was removed by washing three times with PBS, and a swine anti-rabbit peroxidase anti-peroxidase kit (DAKO PAP Kit, k548) was used. The procedures were performed as instructed in the manual of the PAP kit. Two groups of plates were obtained, one receiving PUVA treatment and the other being the control. The plate was read at a wavelength of 405 nm by an ELISA reader (UV max, kinetic microplate reader, Molecular Devices, Menlo Park, CA).

**Selection of Patients and Skin Biopsy** The biopsy specimens were collected from six patients with non-segmental—type vitiligo [18], all of them acrofacial-type vitiligo. The biopsies for all specimens were performed at the dorsa of the hands. All the patients had stable vitiligo, whose lesions had not been enlarging or regenerating and who had not received any treatment in the 3 months prior to biopsy [20]. The biopsies were done at the depigmented lesion immediately before initiation of PUVA treatment and at the repigmented area immediately after a course of PUVA treatment. The PUVA photochemotherapy was performed by ingesting 8-methoxypsoralen (0.6 mg/kg, Star Company, Finland) approximately 2 h prior to UVA irradiation (Dermaray M-DMR-100, Toshiba, Japan). The initial dose of PUVA treatment was 1.0–1.5 J/cm², progressively increased at the interval of 0.5 J/cm² every three treatments of PUVA. Patients received PUVA treatment twice per week, and a 12-week period of treatment was defined as a course of treatment. At the end of a course of treatment, spotty repigmented areas were observed in all six patients.

**Split Dopa Reaction [15] and ATPase Histochemical Reaction [12,21]** The 2-mm punch-biopsied specimens were immersed in 2 N sodium bromide (Sigma) solution at 37°C for 30 min, and then the epidermal sheets were teased off with fine forceps. The sheets were fixed in 5% neutral formalin at 4°C for 20 min; washed in PBS three times; and incubated with either ATP solution or dopa solution.

To demonstrate melanocytes, the sheets were incubated with 0.1% L-dopa in 0.1 M cacodylate buffer (pH 7.4) at 37°C for 3–5 h; they were then washed with cacodylate buffer and examined.

### Table I. EGFR Flow-Cytometry Analysis

<table>
<thead>
<tr>
<th>Dose of PUVA (mj/cm²)</th>
<th>Duration After PUVA for Harvesting</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Immediately (%)</td>
</tr>
<tr>
<td>0 (control)</td>
<td>94 ± 0.7</td>
</tr>
<tr>
<td>31</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>62</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>124</td>
<td>0.3 ± 0.1</td>
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</table>

*The percentage of the total cells counted showing specific FITC-EGFR conjugated fluorescence. The cells, after receiving various dose of PUVA, were collected for analysis immediately, 24 or 72 h after PUVA.

### Table II. Effect of PUVA on Melanocytes Compared with Control, and the Amount of ³H-Thymidine and ¹⁴C-Proline Uptake and Tyrosinase Activity Expressed as Percentage of Control

<table>
<thead>
<tr>
<th>Dose of PUVA (mj/cm²)</th>
<th>³H-Thymidine Uptake</th>
<th>¹⁴C-Proline Uptake</th>
<th>³H₂O Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>31</td>
<td>92.1 ± 4.3%</td>
<td>89.1 ± 3.6%</td>
<td>116.4 ± 5.8%</td>
</tr>
<tr>
<td>62</td>
<td>85.3 ± 5.1%</td>
<td>83.5 ± 3.2%</td>
<td>135.2 ± 7.4%</td>
</tr>
<tr>
<td>124</td>
<td>71.2 ± 5.7%</td>
<td>61.4 ± 3.5%</td>
<td>122.6 ± 6.5%</td>
</tr>
</tbody>
</table>
ATP solution was made up by adding 10 ml 0.125% ATP-Na2 (Sigma), 10 ml 0.2 M Tris-malate (pH 7.2), 2.5 ml 0.1 M MgSO4, and 1.5 ml 2% lead citrate together, and adding distilled water up to 25 ml (pH 7.2). The sheets were incubated with ATP solution at 37°C for 60 min; washed in distilled water three times; and incubated with 1% ammonium sulfide at room temperature for 3–5 min. After being washed, they were examined by a light microscope.

The density of tyrosinase-positive and ATPase-positive cells were counted at six randomly selected fields of each epidermal sheet at the magnification of X100 under a calibrated ocular grid.

Statistical Analysis Triplicate samples from various groups of experiments were analyzed. The data of results represented means ± SD in the whole study. The student t test was used to determine the significance of results. Differences with p < 0.05 were accepted as statistically significant.

RESULTS

Cell Culture and Effect of PUVA on Cell Morphology and Viability In vitro, the melanocytes appeared as bipolar, tripolar, or multipolar dendritic cells after growing in SFM for 25–35 d after primary seeding. There was no significant morphologic change of cells between control groups and experiment groups receiving various doses of PUVA treatment. Considering the cell viability, there was no significant difference between the control group (97.2 ± 3.6%) and the experiment groups receiving 31 mj/cm² (96.5 ± 4.1%), 62 mj/cm² (95.6 ± 4.6%), or 124 mj/cm² (93.5 ± 5.1%).

Effect of PUVA on Expression of Epidermal Growth-Factor Receptor In flow-cytometry analysis of EGFR the autofluorescence (AF) of the background was defined to the left area of the vertical boundary line, and the specific FITC-EGFR conjugated fluorescence was defined as to the right area of the border line. The percentage of total cells counted showing specific FITC-EGFR conjugated fluorescence was determined from all three groups of cells immediately after PUVA (Fig 1), and 24 (Fig 2) or 72 h after PUVA (Table I). There was a significant, PUVA dose-dependent depletion of EGFR expression in cells immediately after PUVA. After 24 h incubation post PUVA, the expression of EGFR significantly reduced in low PUVA dose group (31 or 62 mj/cm²), but not in high PUVA dose group (124 mj/cm²). Seventy-two hours after PUVA, in cells receiving 124 mj/cm² PUVA, the expression of EGFR recovered significantly.

Effect of PUVA on Cell Protein and DNA Synthesis, and Tyrosinase Activity The 3H-thymidine and 14C-proline uptake of cells was significantly decreased in the experimental groups (receiving PUVA) as compared with the control group (Table II); moreover, it showed that the higher the PUVA dose the less the 3H-thymidine and 14C-proline uptake. The tyrosinase activity as revealed by the amount of 3H2O production was significantly higher in the experimental groups than in control groups (Table II); however, the increase of tyrosinase activity was not in accordance with the increasing dose of PUVA. The tyrosinase activity reached a peak when cells received 62 mj/cm² PUVA rather than 124 mj/cm².

Effect of PUVA on VAMA Expression By an indirect immunofluorescence test, 16 of 19 patients' sera (84.2%) showed positive reaction with cultured melanocytes in the control group (Fig 3). However, immediately after 124 mj/cm² PUVA, none of 19 patients' sera revealed a positive reaction. Twenty-four hours after PUVA, 11 of 19 patients' sera (57.8%) showed a positive reaction. Seventy–two hours after PUVA, 13 of 19 patients' sera (68.4%) showed a positive reaction. None of the sera of normal controls showed positive fluorescence deposits in either group of cells with or without PUVA treatment.

The cellular ELISA for detection on VAMA was expressed as the individual optical density reading and shown in Fig 4. In cells incubated with patients' sera, a significantly lower level of optical density (0.135 ± 0.012) was observed in cells immediately after PUVA,

as compared with those without UVA irradiation (0.173 ± 0.016). The optical density was 0.135 ± 0.013 in cells 24 h after PUVA, and 0.151 ± 0.014 in cells 72 h after PUVA. The optical density of cells after incubating with normal sera was 0.078 ± 0.006. The above results revealed that the expression of VAMA was depleted significantly immediately after PUVA, and then progressively recovered after incubation for 24 or 72 h.

Effect of PUVA on Melanocytes and Langerhans Cells in Vitiligo Vulgaris By ATPase histochemical staining, the number of Langerhans cells in epidermal sheets decreased significantly in repigmented area after a course of PUVA (89 ± 12 cells/mm²) as compared with those in non-PUVA-treated depigmented lesions (648 ± 58 cells/mm²) (Fig 5) (Table III). In normal controls, the density of Langerhans cells was 589 ± 46 cells/mm².

By split-dopa reaction, the number of melanocytes in epidermal sheets increased significantly in repigmented area (468 ± 52 cells/mm²) as compared with those in depigmented lesions of stable viti-
ligo (173 ± 34 cells/mm²), whereas, in normal controls, the density of melanocytes was 945 ± 69 cells/mm² (Fig 6) (Table III).

**DISCUSSION**

In this study, we have elucidated several effects of PUVA treatments on normal melanocytes in vitro: 1) the higher the dose of PUVA treatment, the more significant the inhibition of cell DNA and protein synthesis; 2) the higher the dose of PUVA treatment, the more significant the depletion of EGFR expression on cells immediately after PUVA, with the EGFR expression progressively recovering after 24 or 72 h incubation; 3) PUVA stimulated the tyrosinase activity of melanocytes but not in a dose-dependent fashion; and 4) immediately after PUVA, the higher the dose of PUVA, the more significant depletion of VAMA expression on cells with the VAMA expression also progressively recovering after 24 or 72 h incubation. Neither cell viability nor morphologic changes was found to be significantly different between groups receiving PUVA as compared with those without PUVA. In vitiligous vulgaris, it revealed that PUVA depleted epidermal Langerhans cells but stimulated the regrowth of melanocytes in repigmenting areas.

The results of this study show that PUVA cannot only inhibit the cell proliferation but also deplete EGFR expression on melanocytes in vitro, and the results are in accordance with previous reports concerning other culture cell lines [8,9]. How then, in vitiligo, can PUVA stimulate melanocyte proliferation and help repigmentation in vivo? Are the effects of PUVA treatment significantly different between in vivo and in vitro observation? Two possible mechanisms are suggested to be involved in the process of repigmentation of vitiligo vulgaris: first, the PUVA photochemotherapy may help block the melanocyte growth-inhibitory factors (MGIF) present in depigmented lesions of vitiligo patients or, second, the PUVA photochemotherapy may act on epidermal keratinocytes or dermal components to stimulate them to release certain melanocyte growth-stimulatory factors (MGSF) that enhance the proliferation of melanocyte in depigmented lesions. Though the identity of MGSF is unclear at present, MGSF may be a serial compound bearing a biochemical signal for proliferation and include prostaglandins E₂, D₂, etc. [22–24]. Further studies concerning the mechanism of production and action of MGSF are to be done.

Epidermal Langerhans cells are proposed to bear Fc and C₃b receptors [25] and express Ia antigens [26], and may perform functions similar to those of the monocyte-macrophage-histiocyte system, both by initiating the immune response to external antigens and by facilitating an enhanced reaction in the secondary response. In previous reports, epidermal Langerhans cells have been proposed to play a role in the pathogenesis of vitiligo [11,12], and may in depigmented lesions of vitiligo vulgaris inhibit the proliferation of melanocytes by an unknown mechanism. Furthermore, various models of therapies that deplete the number or impair the functions of Langerhans cells may stop further damage to melanocytes and aid in repigmentation. It is reported that large dose of ultraviolet radiation (UVR) and PUVA treatment may deplete the ATPase, OKT6 antigens, HLA-DR antigens, and Fc and C₃b receptors on the surfaces of Langerhans cells [27–29], and thus impair the functions of Langerhans cells in immune reactions. Naughton and colleagues first reported that the majority of individuals with active vitiligo had antibodies to melanocyte-associated antigens [30,31]. Norris et al reported direct evidence that vitiligo patients’ sera containing anti-
Table III. Density of ATPase-Positive and DOPA-Positive Cells at the Depigmented Lesions and Repigmented Areas for Each Patient*

<table>
<thead>
<tr>
<th>Epidermal Sheet</th>
<th>ATPase-Positive Cells/mm²</th>
<th>DOPA-Positive Cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depigmented lesions of stable vitiligo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>625 ± 39</td>
<td>162 ± 12</td>
</tr>
<tr>
<td>Patient 2</td>
<td>681 ± 45</td>
<td>153 ± 16</td>
</tr>
<tr>
<td>Patient 3</td>
<td>595 ± 36</td>
<td>187 ± 15</td>
</tr>
<tr>
<td>Patient 4</td>
<td>720 ± 65</td>
<td>205 ± 18</td>
</tr>
<tr>
<td>Patient 5</td>
<td>711 ± 53</td>
<td>102 ± 9</td>
</tr>
<tr>
<td>Patient 6</td>
<td>505 ± 41</td>
<td>229 ± 17</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>648 ± 58</td>
<td>173 ± 34</td>
</tr>
<tr>
<td>Repigmented areas after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>104 ± 10</td>
<td>531 ± 47</td>
</tr>
<tr>
<td>Patient 2</td>
<td>79 ± 8</td>
<td>423 ± 34</td>
</tr>
<tr>
<td>Patient 3</td>
<td>102 ± 11</td>
<td>437 ± 39</td>
</tr>
<tr>
<td>Patient 4</td>
<td>87 ± 9</td>
<td>512 ± 45</td>
</tr>
<tr>
<td>Patient 5</td>
<td>95 ± 8</td>
<td>517 ± 51</td>
</tr>
<tr>
<td>Patient 6</td>
<td>75 ± 6</td>
<td>388 ± 31</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>89 ± 12</td>
<td>468 ± 52</td>
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</tbody>
</table>

* In normal controls, the density of ATPase-positive cells was 589 ± 46 cells/mm², and the density of DOPA-positive cells was 945 ± 69 cells/mm².

melanocyte antibodies could damage cultured human melanocytes by both complement-mediated damage and antibody-dependent cellular cytoxicity [32]. Epidermal Langerhans cells, bearing the Fc receptor, may be one type of cellular effector able to bind to antimelanocyte antibodies on melanocytes (target cells), to damage the melanocytes and nearby keratinocytes by release of cytoxic products [11,12,20,33]. The released cytoxic products may comprise the majority of MGIF. Furthermore, the C3b receptor on Langerhans cells may also promote the lysis of C3b-bearing melanocytes. Therefore, PUVA treatment, as used in vitiligo vulgaris, may not only deplete the VAMA on melanocytes, but may also deplete the Fc and C3b receptors and HLA-DR antigens on the surfaces of Langerhans cells. All the above reactions may help block the pathways of pathogenesis of vitiligo at certain points including: 1) blocking the binding of antimelanocyte antibodies to VAMA on melanocytes; 2) blocking the participation of Langerhans cells in antibody-dependent cytoxicity; and 3) blocking the antigen-presenting ability of Langerhans cells.

In this study, PUVA treatment depleted the expression of VAMA and EGFR in a dose-dependent fashion. Although the precise mechanism of PUVA photoreaction is unknown, it may act on cell membrane directly or indirectly. PUVA treatment may directly damage membrane glycoproteins, which results in alternation of the cell membrane markers or receptors, or it may indirectly alter the cellular metabolism, resulting in a decrease of antigenicity or of receptors on the cell membrane [34,35]. However, the depletion of EGFR and VAMA expression was most significant immediately after PUVA, and progressively recovered after 24 or 72 h incubation. It is proposed that the major photoreaction may act directly on cell membranes by altering the structures of EGFR and VAMA rather than indirectly altering the central metabolic system.

In summary, PUVA treatment in vitro inhibits melanocyte proliferation, enhances tyrosinase activity, and depletes EGFR and VAMA on the cell membrane of melanocytes. However, in vivo, PUVA treatment promotes melanocyte repopulation of depigmented lesions of vitiligo vulgaris. Two possibilities are proposed to explain how PUVA treatment works in vitiligo. First, PUVA treatment may stimulate the production of MGF by other components of skin, which play a role in promoting melanocyte proliferation in the depigmented lesions. Second, PUVA treatment may deplete the VAMA of melanocytes and also impair the functions of Langerhans cells in immune reactions, and thus block the damage to melanocytes.

Figure 6. The number of melanocytes in epidermal sheets decreased significantly in depigmented lesions (173 ± 34 cells/mm²) (B) as compared with those in normal skin (945 ± 69 cells/mm²) (A). In the repigmented skin, the return of melanocytes were observed (468 ± 52 cells/mm²) (C). Split-dopa reaction. Magnification × 100.

REFERENCES


ANNOUNCEMENT

We are pleased to announce that an International Symposium entitled “Skin Therapy Forum —A Future Look” will be held in the University of Wales College of Medicine, Cardiff, on October 25–28, 1992. The meeting is being organized by Professor R Marks of Cardiff and Dr W J Cunliffe of Leeds and is a British Association of Dermatologists sponsored event.

The programme will consist of invited lectures, free communications, poster papers, and lunchtime debates. The following sessions will be included: New Molecules for Old Diseases; New Indications for Old Molecules; Principles, Practice and Ethics of Clinical Trials; Surgical Treatment; and Management of Patients with Difficult Diseases. It is planned that the proceedings of the meeting will be published.

Abstract forms and further details may be obtained from Mrs Joy Hayes, Department of Dermatology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, United Kingdom.