Psoralens potentiate ultraviolet light-induced inhibition of epidermal growth factor binding

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ABSTRACT The psoralens, when activated by ultraviolet light of 320–400 nm (UVA light), are potent modulators of epidermal cell growth and differentiation. Previously, we reported that, in mammalian cells, these compounds bind to specific saturable high-affinity cellular receptor sites. In the present studies, we demonstrate that binding of psoralens to their receptors followed by UVA light activation is associated with inhibition of epidermal growth factor (EGF) receptor binding. Inhibition of EGF binding, which required UVA light, was rapid and dependent on the dose of UVA light (0.5–2.0 J/cm²), as well as the concentration of psoralens (10 nM to 1 μ M). Higher doses of UVA light (2.0–6.0 J/cm²) by themselves were also inhibitory, indicating that psoralens potentiate the UVA-induced inhibition of EGF binding. A number of biologically active analogs of psoralen, including 8-methoxypsoralen, 5-methoxypsoralen, and 4,5',8-trimethylpsoralen, when activated by UVA light, were found to be inhibitors of binding. Inhibition of EGF binding by psoralens was observed in a variety of human and mouse cell culture lines known to possess psoralen receptors. In the epidermal-derived line PAM 212, at least two populations of receptors with different affinities for EGF were found. Psoralens and UVA light selectively inhibited binding to the higher-affinity EGF receptors, an effect analogous to that of the phorbol ester tumor promoters. As observed with phorbol esters, photoactivated psoralens appeared to inhibit EGF binding by an indirect mechanism. These data demonstrate that the psoralens and UVA light have direct biological effects on cell-surface membranes. Since EGF is a growth-regulatory peptide, the ability of psoralens and UVA light to inhibit EGF binding may underlie the biologic effects of these agents in the skin.

Ultraviolet light irradiation alone and in combination with chemical photosensitizers, such as psoralens, has considerable biological activity in the skin, causing erythema, sunburn, and tanning, as well as inducing alterations in epidermal cell growth and differentiation (1, 2). This biological activity is useful in the treatment of a number of skin disorders, including psoriasis, vitiligo, mycosis fungoides, and eczema (3). The predominant wavelengths of ultraviolet light in sunlight, and those used in photomedicine, are in the range of 320 to 400 nm (UVA light) and 290 to 320 nm (UVB light). Although it has been postulated that the generation of intracellular free radicals and DNA damage underlies the biological effects induced by ultraviolet light in the skin, the precise molecular and cellular mechanisms involved are not known (4, 5).

We have been interested in elucidating the mechanism of action of UVA light and psoralens in the skin. Psoralens, also known as furocoumarins, are plant-derived compounds that can intercalate into cellular DNA in a dark reaction. After exposure to UVA light, these compounds can form adducts

with pyrimidine bases in the DNA. Recently, we have shown that mammalian cells possess specific high-affinity receptor sites for psoralens (6). These receptors are distinct from the DNA, are localized in the cytoplasm and cell membranes, and can be alkylated by psoralens following UVA light treatment. In addition, there is a direct correlation between the binding of several psoralen analogs to these receptors and their biological activity in skin photosensitization assays. Since $\approx 80\%$ of psoralen in treated cells is receptor-associated, we have hypothesized that skin photosensitivity reactions are mediated, at least in part, by UVA light activation of the psoralen–receptor complex.

In the present paper, these observations have been extended by demonstrating that psoralens bound to mammalian cells and activated by UVA light have direct biological effects on the cell-surface membrane. We report that UVA light is a potent inhibitor of epidermal growth factor (EGF) receptor binding, and that this effect is potentiated by psoralens. Inhibition of EGF receptor binding by psoralens and UVA light is rapid and irreversible. The EGF receptor is a tyrosine-specific protein kinase whose activity is known to be important in the regulation of cellular proliferation (7). Since epidermal cell growth is modulated by UVA light and psoralens, interaction with EGF receptors in the skin may underlie their mechanism of action.

MATERIALS AND METHODS

Reagents. 8-Methoxypsoralen, 5-methoxypsoralen, and 4,5',8-trimethylpsoralen (TMP) were obtained from Elder Pharmaceuticals (Bryant, OH). Psoralen and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma. ¹²⁵I-labeled EGF (¹²⁵I-EGF) (specific activity, 200 Ci/g; 1 Ci = 37 GBq) was from ICN. Unlabeled EGF was from Collaborative Research (Waltham, MA). 8-[methoxy-³H]Methoxypsoralen (67.9 Ci/mmol), 12,13-[20-³H(N)]phorbol dibutyrate ([³H]PBT₂) (13.4 Ci/mmol), ¹²⁵I-labeled insulin (A chain ¹²⁵I-labeled tyrosine-14) (2200 Ci/mmol), and L-[4,5-³H(N)]leucine were obtained from New England Nuclear.

Cell Cultures. All cell cultures, including HeLa, HEP-2, KB, S-180, B16, and PAM 212 were obtained as described (6). Cells were maintained in monolayer culture at 37°C in humidified 5% CO₂/95% air in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum.

Binding Assays. Unless otherwise specified, all binding assays were performed at 4°C. Cells were inoculated into 60-mm culture dishes (1.8 $\times 10^4$ cells per cm²) and incubated

Abbreviations: UVA light, ultraviolet light of wavelength 320–400 nm; UVB light, ultraviolet light of wavelength 290–320 nm; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor; ¹²⁵I-EGF, ¹²⁵I-labeled EGF; 8-MOP, 8-methoxypsoralen; 5-MOP, 5-methoxypsoralen; TMP, 4,5',8-trimethylpsoralen; PBT₂, phorbol 12,13-dibutyrate; psoralen/UVA, psoralens photoactivated by UVA light.

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at 37°C. After 4–5 days, the cells were washed three times with 2 ml of binding buffer (DMEM/0.1% bovine serum albumin/25 mM Hepes, pH 7.2) and then incubated for 2 hr at 4°C with 2 ml of buffer containing 2 nM 125 I-EGF (200 Ci/g). Nonspecific binding was determined by incubating the cells with buffer containing the radioligand and excess unlabeled EGF (1 μ g/ml). The reaction was terminated by aspirating the binding buffer from the culture dishes and washing the cells four times with 5 ml of ice-cold phosphate-buffered saline (PBS). The cells were then solubilized with 2 ml of 0.2 M NaOH and duplicate 0.5-ml aliquots were taken for γ counting. Specific binding was calculated by subtracting nonspecifically bound material from the total. The data are presented as the average of duplicate culture plates. Under the conditions used, specific binding represented at least 70–80% of the total bound in each of the cell lines used. To determine saturability, the assay was performed with binding buffer containing increasing concentrations of 125 I-EGF. The effects of UVA light and psoralens on EGF receptor number and affinity were analyzed according to the method of Scatchard (8). Curvilinear Scatchard plots for PAM 212 epidermal cells were analyzed in terms of two independent binding sites (9). Specific binding of 8- 3 H)methoxypsoralen was determined as described (6). Phorbol ester binding was assayed as described by Dunn *et al.* (10) using [3 H]PBT₂ as the radioligand. 125 I-labeled insulin receptor binding was assayed by the method of Raizada *et al.* (11).

Treatment of Cells with Psoralens and UVA Light. Cells were washed three times with PBS and then incubated with psoralens in 2 ml of Eagle's salt solution supplemented with 5.2 mM D-glucose/25 mM Hepes, pH 7.2. Control cultures were incubated in 2 ml of Earle's salt solution in the absence of psoralens. The cells were then exposed to UVA light emitted from a bank of four BLB fluorescent light tubes (F40 BL, Sylvania) placed $\approx$ 10 cm above the cell culture plates. The incident light on the culture plates ranged from 3.4 to 6.8 mW per cm² of UVA light and was measured with an International Light UV-radiometer (model 442) fitted with an IL-SE 115 probe and 363 UVA pass filter. Typical exposure times to UVA light ranged from 2.5 to 10 min. After light exposure, cells were rinsed three times with binding buffer and used immediately in receptor-binding assays. In some experiments, cells were pretreated for 30 min with the tumor promoter PMA prior to 125 I-EGF receptor binding assays. To monitor recovery of 125 I-EGF receptor binding following exposure to PMA, UVA light, and psoralen, treated cells were washed two times with 2 ml of binding buffer and then incubated at 37°C with 2 ml of fresh growth medium for increasing periods of time up to 4 hr. Cells were then rinsed three times with PBS and assayed for EGF receptor binding as described above.

Treatment of the cells with psoralens and/or UVA light had no effect on the attachment of the cells to the culture dishes or on cell viability, as determined by trypan blue dye exclusion. [3 H]Leucine uptake into cellular proteins was also not affected by drug treatment during the course of the experiments. In all experiments, control dishes were protected from UVA light exposure by an opaque covering.

RESULTS

UVA Light Inhibits Specific Binding of 125 I-EGF Binding to Mammalian Cells. Initially, we examined the binding of 125 I-EGF to a number of different mouse and human cell lines in culture. These cell lines were chosen for study because we have previously shown that they possess specific high-affinity receptors for the psoralens (6). Specific binding of 125 I-EGF could be detected in each of the cell lines examined except the B16 (Table 1). Pretreatment of the cell lines with UVA light was found to inhibit binding of 125 I-EGF to its

Table 1. Inhibition of 125 I-EGF-specific binding after exposure of different cell lines in culture to UVA light and psoralens

Cell line	125 I-EGF-specific binding, cpm per 10 ⁶ cells			
	Control	TMP	UVA light	UVA light + TMP
Human				
HeLa	2390	2356 (2)	2223 (7)	581 (76)
KB	9303	9390 (—)	5396 (42)	3465 (63)
Hep-2	2302	2271 (1)	1888 (18)	907 (61)
Mouse				
PAM 212	4152	4122 (0)	1827 (56)	501 (88)
S-180	5104	4916 (4)	2756 (46)	1178 (81)
B16	NB	NB	NB	NB

Cells grown in 60-mm culture dishes were incubated in binding buffer with and without TMP (4.4 μ M). After 30 min, some of the cultures were exposed to UVA light (4.1 J/cm²) and then assayed for specific binding of 125 I-EGF as described. Nonspecific binding was determined by adding excess unlabeled EGF (1 μ g/ml) to the binding buffer. This amount was subtracted from total 125 I-EGF binding to obtain the amount of 125 I-EGF specifically bound to the cells. Numbers in parentheses represent the percentage inhibition of specific 125 I-EGF binding compared to controls. Note that psoralens potentiated the UVA light-induced inhibition of 125 I-EGF binding. NB, no binding.

membrane receptors. In each of the cell lines examined, inhibition of EGF binding was evident immediately following UVA light treatment. UVA light had no effect on nonspecific binding of EGF (not shown). Using PAM 212 and HeLa cells, inhibition of 125 I-EGF binding by UVA light was found to be dose dependent in the range of 0.5–6.0 J/cm² (Fig. 1). PAM 212 cells were 2–3 times more sensitive than HeLa cells to the inhibitory effects of UVA light on EGF binding.

Psoralens Potentiate the Effects of UVA Light on 125 I-EGF Receptor Binding. When the different cell lines were incubated with the psoralen analog TMP (4.4 μ M), prior to UVA light, a potentiation of the UVA light-induced inhibition of 125 I-EGF binding was observed (Table 1). This potentiation varied with the cell type and ranged from 20% to 70%. Interestingly, in PAM 212 cells, the combination of UVA light and TMP, or another psoralen analog, 8-methoxypsoralen was more effective at inhibiting EGF binding than the potent phorbol ester tumor promoter PMA, a well-characterized inhibitor of EGF binding (Table 2) (12). Treatment of PAM 212 cells with TMP following UVA light produced no further inhibition of 125 I-EGF binding than with UVA light alone (not shown). In addition, neither TMP alone (Tables 1

FIG. 1. Inhibition of 125 I-EGF specific binding by UVA light and psoralens varies with the dose of UVA light. HeLa (A) and PAM 212 cells (B) grown in 60-mm culture dishes were exposed to increasing doses of UVA light with and without TMP pretreatment and then assayed for specific binding of 125 I-EGF as described. ■, UVA light alone; ▲, TMP (44 nM) plus UVA; ♦, TMP (0.44 μ M) plus UVA; ●, TMP (4.4 μ M) plus UVA. Note that greater doses of UVA light were required to inhibit EGF binding in HeLa cells when compared to the PAM 212 cells.

Table 2. Selective inhibition of ^{125}I -EGF binding to PAM 212 epidermal cells by ultraviolet light with and without psoralen treatment

Cell treatment	% inhibition of specific binding	
	^{125}I -EGF	$[^3\text{H}]\text{PBT}_2$
TMP (4.4 μM)	0	2
8-Methoxypsoralen (4.6 μM)	0	1
PMA (0.17 μM)	69	64
UVA (1.4 J/cm 2)	4	1
UVA (2.8 J/cm 2)	17	2
UVA (1.4 J/cm 2) +		
8-Methoxypsoralen (46 nM)	24	ND
8-Methoxypsoralen (0.46 μM)	61	ND
8-Methoxypsoralen (4.6 μM)	93	0
UVA (1.4 J/cm 2) +		
TMP (44 nM)	44	ND
TMP (0.44 μM)	90	ND
TMP (4.4 μM)	93	1
UVA (1.4 J/cm 2) +		
Psoralen (5.4 μM)	84	2
5-Methoxypsoralen (4.6 μM)	49	0

PAM 212 cells grown in 60-mm culture dishes were treated with 2 ml of binding buffer with and without psoralen or PMA as indicated. After 30 min, the cells were pulsed with UVA light and then assayed for specific binding of ^{125}I -EGF or the phorbol ester $[^3\text{H}]\text{PBT}_2$. Note that photoactivated psoralens specifically inhibit ^{125}I -EGF binding. ND, not determined.

and 2) nor TMP treated with UVA light prior to its addition to the cells (not shown) inhibited EGF binding, indicating that psoralen receptor binding and activation by UVA light were required for inhibition to occur. As observed with UVA light alone, TMP plus UVA light had no effect on nonspecific EGF binding in the cells (not shown). In PAM 212 and HeLa cells, the potentiation of UVA light-induced inhibition of ^{125}I -EGF binding by TMP was dose dependent in the concentration range of 10 nM to 1 μM (Table 2; Fig. 1). From these data, it is apparent that, in the presence of TMP, lower doses of UVA light were required to inhibit ^{125}I -EGF binding. We also found that the epidermal cell line, PAM 212, was more sensitive than the HeLa cells to the effects of equivalent doses of TMP plus UVA light (Figs. 1 and 2). This is similar to the results we observed with UVA light alone. Fig. 2 shows that ≈ 70 times more TMP is required to potentiate the inhibition of EGF binding in HeLa cells than in PAM 212 cells.

In addition to TMP, several active skin photosensitizing analogs, including psoralen, 8-methoxypsoralen, and 5-methoxypsoralen potentiated the UVA light-induced inhibi-

Fig. 2. Inhibition of ^{125}I -EGF binding by TMP and UVA light varies with the concentration of TMP. Cells were pretreated for 30 min with increasing concentrations of TMP and then pulsed with UVA light (4.1 J/cm 2) as described. Control cells were treated with UVA only; ■, HeLa cells; ●, PAM 212 cells. Note that PAM 212 cells were more sensitive to psoralen/UVA.

tion of ^{125}I -EGF receptor binding (Table 2). These analogs were effective inhibitors in the concentration range of 0.1–10 μM and UVA light at 1.4 J/cm 2 . Of the analogs tested, TMP was the most potent inhibitor, followed by psoralen, 8-methoxypsoralen, and 5-methoxypsoralen.

^{125}I -EGF receptor binding was not inhibited by the psoralen analogs 8-methoxypsoralen and TMP in competitive binding assays (Table 3). Furthermore, EGF (1 $\mu\text{g}/\text{ml}$) did not compete with psoralens for receptor binding (not shown). This suggests that psoralens do not compete directly with EGF for its receptor. We also examined the effects of psoralens on phorbol ester receptor binding. We have previously shown that PMA does not compete with the psoralens for receptor binding (6). Similarly, TMP and 8-methoxypsoralen did not inhibit $[^3\text{H}]\text{PBT}_2$ binding regardless of whether the cells were pretreated or coincubated with the psoralens or when the psoralens were activated by UVA light (Tables 2 and 3). UVA light alone also had no effect on phorbol ester receptor binding (Table 2). In contrast, PMA inhibited both ^{125}I -EGF and $[^3\text{H}]\text{PBT}_2$ binding, while EGF inhibited only ^{125}I -EGF binding (Table 2). These data indicate that inhibition of EGF binding by UVA light and psoralens occurs by a mechanism that is distinct from the phorbol ester tumor promoters.

We also tested the effects of TMP and UVA light on ^{125}I -labeled insulin receptor binding in PAM 212 cells. Neither TMP (4.4 μM), UVA light (2.8 J/cm 2), nor the combination of TMP and UVA light inhibited insulin receptor binding to the cells (not shown). These data, together with our findings that these treatments had no effect on $[^3\text{H}]\text{PBT}_2$ binding, suggest that the biological effects of psoralens and UVA light are specific for the EGF receptor.

Characterization of ^{125}I -EGF Binding in PAM 212 Cells. The saturation of EGF binding sites in PAM 212 epidermal cells was examined by incubating the cells in the presence of increasing concentrations of ^{125}I -EGF with and without prior treatment with UVA light alone, or UVA light plus TMP. Scatchard analysis of the binding data in untreated cultures produced curvilinear plots (Fig. 3), which were similar to those reported for primary mouse epidermal cultures (9). These curves were interpreted in terms of two completely independent binding sites (Fig. 3 *Inset*): a higher-affinity binding site with a dissociation constant (K_d) of 33 pM and 1200 binding sites per cell and a lower-affinity site with a K_d of 3.1 nM and 25,000 sites per cell. Pretreatment of the cells with UVA light (2.0 J/cm 2) specifically decreased both the number and affinity of the higher-affinity binding sites for EGF (Fig. 3). Thus, following UVA light, the K_d increased to 66 pM and the number of higher-affinity receptors decreased to 960 sites per cell (graphic analysis not shown). Although UVA light caused a small increase in the K_d of the lower-affinity sites, there was no effect on the number of these sites. Incubation of PAM 212 cells with TMP (4.4 μM) prior to the

Table 3. Effect of psoralen analogs, PMA, and EGF on receptor binding in PAM 212 cells

Addition to binding assay	Total binding, cpm per 10 6 cells	
	^{125}I -EGF	$[^3\text{H}]\text{PBT}_2$
None	5036	2703
TMP (4.4 μM)	4908 (3)	2549 (6)
8-Methoxypsoralen (4.6 μM)	5308 (0)	2852 (0)
PMA (0.17 μM)	1964 (61)	1048 (61)
EGF (1 $\mu\text{g}/\text{ml}$)	1484 (73)	2469 (9)

EGF and phorbol ester binding assays were performed at 37°C as described. Psoralens, PMA, and EGF were coincubated with the radioligand. Numbers in parentheses represent the percentage inhibition of ^{125}I -EGF binding compared to untreated controls. Note that TMP and 8-methoxypsoralen did not inhibit ^{125}I -EGF or $[^3\text{H}]\text{PBT}_2$ binding.

FIG. 3. Scatchard analysis of ^{125}I -EGF binding in PAM 212 cells with and without UVA and TMP/UVA treatment. Saturation of specific binding of ^{125}I -EGF was determined with increasing concentrations of ^{125}I -EGF. Cells were treated with UVA (4.1 J/cm²) or TMP (4.4 μM) and UVA (4.1 J/cm²) immediately prior to the ^{125}I -EGF binding assay. ●, Control; ▲, UVA; ◆, TMP and UVA. (Inset) Graphic resolution of Scatchard plot. The curvilinear Scatchard plot from control cells was resolved by graphic analysis into two straight lines representing two classes of receptors with different affinities (dotted lines).

UVA light potentiated the inhibition of ^{125}I -EGF, nearly eliminating high-affinity EGF receptor binding (Fig. 3).

Lack of Recovery of ^{125}I -EGF Binding in PAM 212 Cells Following Psoralen and UVA Light Treatment. Previous studies have shown that inhibition of EGF receptor binding by phorbol esters is reversible (11). In PAM 212 cells, we observed complete recovery of binding within 1 hr following exposure to 5 nM PMA, a concentration that produces a 42% inhibition of ^{125}I -EGF binding (Fig. 4). In contrast, under the experimental conditions used, ^{125}I -EGF receptor binding did not recover in the cells following treatment with UVA light alone (2.0 J/cm²), or in combination with TMP (4.4 μM) (Fig. 4). This is particularly interesting since all of the treatments tested lead to rapid inhibition of EGF binding. The fact that the kinetics of recovery are dissimilar provides further evidence that photoactivated psoralens and phorbol esters inhibit EGF binding by distinct mechanisms.

DISCUSSION

In 1978, Lee and Weinstein (12) demonstrated that tumor-promoting phorbol esters specifically inhibited binding of EGF to its cell-surface receptors. Inhibition of EGF binding

FIG. 4. Recovery of ^{125}I -EGF specific binding in PAM 212 cells following inhibition by PMA, UVA, and TMP/UVA treatment. PAM 212 cells grown in 60-mm culture dishes were treated with 5 nM PMA (●), UVA at 4.1 J/cm² (■), 4.4 μM TMP and UVA at 4.1 J/cm² (▲), rinsed, and then incubated in 2 ml of drug-free growth medium at 37°C for increasing periods of time before being assayed for ^{125}I -EGF binding as described.

by phorbol esters was rapid and did not occur by competition, but through an indirect mechanism (13–15). The rationale for examining the effects of PMA on EGF binding was based on the fact that tumor promoters and EGF shared a number of similar biological effects on mammalian cells. It has now been established that PMA binds to protein kinase C, and not directly to the EGF receptor, and that this enzyme, when stimulated by phorbol esters, can directly phosphorylate the EGF receptor. This process induces changes in the EGF receptor in the cells, resulting in decreased EGF binding (15, 16).

The psoralens when activated by ultraviolet light and, to some extent, ultraviolet light alone share a number of biological properties with EGF and the phorbol esters. These include the ability to regulate epidermal cell proliferation and keratinization (17) and to induce epidermal ornithine decarboxylase (18, 19). Psoralens and UVA light (psoralen/UVA), like the phorbol esters, have also been reported to induce melanogenesis in pigment-producing cells in culture (20, 21). In addition, evidence suggests that psoralen/UVA may be a cocarcinogen (22). These findings, together with the fact that both psoralens and phorbol esters have specific high-affinity cellular receptors, prompted us to investigate whether psoralen/UVA also modulates EGF receptor binding. In this paper, evidence is presented that psoralens bound to mammalian cells and subsequently activated by low doses of UVA light (0.5–2.0 J/cm²) are, in fact, potent inhibitors of EGF receptor binding. High doses of UVA light (2.0–6.0 J/cm²) alone are also inhibitory. Inhibition of EGF binding by psoralens plus UVA light, as well as UVA light alone, is rapid and dose dependent. Furthermore, several biologically active psoralen analogs, including TMP, psoralen, 8-methoxypsoralen, and 5-methoxypsoralen, when activated by UVA light also inhibit EGF binding. These data demonstrate that photoactivated psoralens have direct biological actions on the cell-surface membrane.

Both UVA light and psoralen/UVA were found to inhibit EGF binding in a number of different cell lines, including HeLa, KB, Hep-2, S-180, and PAM 212 cells. We have previously shown that these cells possess high-affinity binding sites for the psoralens, suggesting an association between psoralen receptor binding and their effects on EGF receptors (see below). Inhibition of EGF binding in these different cell lines appeared to vary with their tissue origin. For example, PAM 212, which is an epidermal-derived cell line, was ≈ 3 times more sensitive to UVA light and required 70 times less TMP to inhibit EGF binding than the human carcinoma cell line HeLa. The greater sensitivity of epidermal cells to UVA light and psoralen/UVA treatment may underlie the clinical responsiveness of skin cells to these drugs. Although PAM 212 cells were more sensitive to inhibition of EGF binding by psoralen/UVA, these cells in fact bound less psoralen than the HeLa cells (6). We have previously described both higher- and lower-affinity psoralen receptors in HeLa and PAM 212 cells. At present, it is not known which of these receptor populations in each cell type is responsible for the biological effects observed.

We also observed that the B16 cell line did not bind ^{125}I -EGF. We have previously demonstrated that these cells possess high-affinity receptors for psoralens (6). The apparent lack of EGF receptors on these cells suggests that psoralen action on melanocyte-derived cells is mediated by a mechanism distinct from EGF binding. This is not surprising since psoralens exert opposing effects on epidermal keratinocytes and melanocytes (23).

The inhibitory effects of psoralen/UVA and UVA on receptor binding appeared to be specific for EGF, as evidenced by the fact that these compounds had no effect on phorbol ester or insulin binding. The identity of the chromophore that absorbs UVA light in cells and causes

decreased EGF receptor binding is unknown. It has been established that UVA light can interact directly with nucleic acids (24). In the present studies, the addition of psoralens to the cells was found to enhance the effects of UVA light on EGF binding. This suggests that UVA light alone acts at the level of the psoralen receptor, although UVA light has not been found to interfere with the binding of psoralen to its receptor (unpublished studies). We also cannot rule out that UVA light acts directly on the EGF receptor, on protein kinase C, or some other membrane component. Previous studies have shown that UVB light inhibits specific binding of EGF to cultured murine fibroblasts (25) and prostaglandin E_2 to membrane preparations of human skin (26). The mechanism underlying the effects of UVB light on EGF receptor binding is not known. UVA fluorescent bulbs emit low doses of UVB irradiation. Thus, UVB light may contribute to the inhibition of EGF binding induced by UVA light alone.

The effects of psoralen/UVA and PMA on EGF binding were found to be similar, although not identical. Both psoralen/UVA and PMA inhibited >85% of EGF receptor binding in PAM 212 cells. The fact that psoralen/UVA-induced inhibition occurred only after the cells were treated suggests that the drug is not simply competing for EGF binding. This is supported by our findings that psoralens, while binding to their own receptors in PAM 212 cells, did not compete directly with EGF in receptor binding assays. Conversely, EGF also failed to displace psoralen from its receptor sites. These data suggest that the effects of psoralens on EGF receptor binding, like the phorbol esters, are initiated by an indirect mechanism (13, 14). Both psoralen/UVA and PMA selectively affected higher-affinity EGF receptor sites. We found that preincubation of PAM 212 cells with psoralen/UVA significantly altered both the apparent K_d and the number of higher-affinity EGF receptor sites. UVA light alone also inhibited higher-affinity EGF binding. It has previously been reported that PMA selectively inhibits higher-affinity EGF receptor sites in RAT-1 and primary mouse epidermal cells (9, 27).

The major difference between psoralen/UVA and tumor promoters on EGF binding was in the kinetics of recovery from inhibition. Within 1 hr after PMA treatment of PAM 212 cells, EGF receptor binding had returned to control levels. However, both UVA and psoralen/UVA-induced inhibition of EGF binding were found to persist in the cells for at least 4 hr. The mechanism by which psoralens and UVA light inhibit recovery of EGF binding in the PAM 212 cells is not known. However, these data, together with our findings that PMA and psoralens did not compete with one another for receptor binding, suggest that the two agents inhibit EGF binding by different mechanisms.

Treatment of the skin with psoralens and ultraviolet light initiates a complex series of biological events. In a previous publication, we demonstrated that mammalian cells, including those derived from the skin, possess specific high-affinity receptor sites for the psoralens (6). Based on this observation, we hypothesized that the biological responses induced by these compounds were due to specific psoralen binding followed by UVA light activation of the psoralen bound to its receptor. We now suggest that decreased EGF binding is one of the biological responses resulting from psoralen-receptor photoactivation. At higher doses, UVA light alone also decreased EGF binding but its mechanism may be different than that of the photoactivated psoralens. The EGF receptor is a tyrosine-specific protein kinase that is known to be

involved in cell-growth regulation (7). Psoralen/UVA or UVA light-induced changes in the kinase activity of this receptor may initiate a cascade of biochemical events leading to cellular responses. In this regard, PMA-induced inhibition of EGF binding is associated with decreased EGF receptor kinase activity (15). Modulation of cellular growth-factor receptors other than EGF or the stimulation of the production of intracellular growth regulatory signals by the activated psoralen receptor may also mediate the biological activity of the psoralens and UVA light. One could speculate that the psoralens and UVA light affect the EGF receptor by activating protein kinase C, possibly by stimulating the production of diacylglycerol, an endogenous activator. Monitoring the levels of this or other potential activators in treated cells may provide important clues in understanding the mechanism of action of the psoralens and UVA light.

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