Biochemical Pharmacology, Vol. 41, No. 1, pp. 125–132, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

PSORALEN BINDING AND INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING BY PSORALEN/ ULTRAVIOLET LIGHT (PUVA) IN HUMAN EPITHELIAL CELLS

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(*Received* 6 *July* 1989; *accepted* 9 *August* 1990)

Abstract—The psoralen analogs 8-methoxypsoralen (8-MOP) and 4,5′,8-trimethylpsoralen (TMP), in combination with ultraviolet light (UVA, 320–400 nm), are potent modulators of epidermal cell growth and differentiation and are commonly used in photochemotherapy of psoriasis and vitiligo. We have used KB cells, a human epithelial cell line, to examine the mechanism of action of these compounds. In KB cells, 8-MOP was found to bind to specific, saturable receptor sites. Binding of [^{3}H]-8-MOP to its receptor was inhibited by TMP as well as psoralen. We found that binding of these analogs to the cells followed by UVA light treatment was associated with inhibition of epidermal growth factor (EGF) receptor binding. Inhibition of EGF binding was temperature dependent, occurred immediately following UVA light exposure, and appeared to be due to a decrease in the number of EGF receptors. In KB cells, ^{125}I-labeled EGF surface receptor binding is followed by its rapid internalization and degradation. We found that photoactivated psoralens also inhibited internalization of ^{125}I-EGF, but had no apparent effect on EGF metabolism. These data indicate that the cell surface membrane may be an important target for the photoactivated psoralens. In addition, since photoactivated psoralens regulate cell proliferation, the interaction of these compounds with EGF receptor function may underlie their biological activity.

The psoralens are a family of substituted furocoumarins that have been used in photochemotherapy for a number of skin disorders including psoriasis and vitiligo. In typical treatment protocols, patients are given 8-methoxypsoralen (8-MOP) or 4,5′,8-trimethyl-psoralen (TMP), and then exposed to a measured dose of long wave ultraviolet light (UVA, 320–400 nm), a procedure referred to as PUVA chemotherapy. This results in an inhibition of the abnormally high rates of keratinocyte proliferation and differentiation associated with psoriasis [1]. Interestingly, PUVA therapy also stimulates melanocyte growth and melanin production resulting in skin tanning. This latter effect has been useful in the treatment of the leukodermic patches observed in vitiligo [2]. An important question from these clinical observations is how PUVA can have such divergent effects on growth and differentiation of different skin cell populations.

We have shown previously that psoralens bind to specific receptor sites located in the cytoplasm and cell membranes of responsive cell types [3, 4]. Binding of psoralens to these receptors is reversible, saturable, and of high affinity. This receptor is independent of the DNA and has been found to be a protein with a molecular mass of 22,000 daltons [4]. Since structurally related psoralen analogs bind to this receptor in a manner that parallels their biological activity, we hypothesize that activation of these receptors by photoactivated psoralens mediates, at least in part, the biological actions of these compounds on the skin [3]. In support of this model, we have demonstrated that psoralens exert direct actions on the cell surface membrane. When activated by ultraviolet light, psoralens are potent inhibitors of epidermal growth factor (EGF) receptor binding [5]. Inhibition of EGF binding is rapid and occurs by an indirect mechanism in a manner analogous to the phorbol ester tumor promoters [6]. The EGF receptor is a tyrosine-specific protein kinase known to be involved in cell growth regulation [7]. Since psoralens modulate epidermal cell growth, the ability of these compounds to interact with the EGF receptor may underlie the cellular mechanism of action of these compounds in the skin. This is supported by recent findings demonstrating that there are changes in EGF binding and receptor distribution in psoriatic epidermis [8] as well as alterations in the expression of transforming growth factor alpha, a structural analog of EGF which binds to the EGF receptor [9].

In the present studies, we characterized psoralen receptor binding to KB epithelial cells and analyzed the effects of UVA light activation of these compounds on EGF binding, internalization and metabolism. KB cells are derived from a human epithelial carcinoma. These cells possess large numbers of both psoralen and EGF receptors [3, 5]. Internalization and degradation of EGF in these cells have been characterized previously [10]. We found that, in KB cells, psoralen photoactivation results in inhibition of EGF binding and internalization. However, there was no apparent effect on degradation of the internalized receptor–ligand

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complex. These results are consistent with our model that psoralens exert their actions by interacting with components of the cell membrane.

MATERIALS AND METHODS

Reagents and cell cultures. 8-MOP, 5-methoxypsoralen (5-MOP) and TMP were obtained from Elder Pharmaceuticals (Bryant, OH). Psoralen and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were obtained from the Sigma Chemical Co. (St. Louis, MO). ^{125}I-labeled EGF (sp. act. 200 Ci/g) was obtained from ICN (Irvine, CA). Unlabeled EGF was from Collaborative Research (Bedford, MA). 8-[*methoxy*-^{3}H]Methoxypsoralen ([^{3}H]-8-MOP) (67.9 Ci/mmol) was purchased from New England Nuclear (Boston, MA). KB cells (M.A. Bioproducts, Walkersville, MD) were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum.

Radioligand binding assays. [^{3}H]-8-MOP and ^{125}I-EGF receptor binding assays were performed in 6-cm plastic petri dishes as previously described with some modifications [3, 5]. Briefly, to assay psoralen binding, cells (approximately 3×10^{6}) were incubated with 2 mL of HEPES (25 mM, pH 7.2) buffered Earle's saline supplemented with D-glucose (5.2 mM) and [^{3}H]-8-MOP (2.4 nM, 67.9 Ci/mmol). After 30 min at 4°, the reaction was terminated by rinsing the cells rapidly with ice-cold phosphate-buffered saline (PBS) (5×5 mL) followed by solubilization in 2 mL of 0.2 N NaOH. Duplicate 0.5-mL aliquots were then counted on an LKB scintillation counter. In competitive binding assays, increasing concentrations of various psoralens were added directly to the incubation mixture. Scatchard analysis was performed in the presence of increasing concentrations of labeled 8-MOP. In these studies, we found that binding of [^{3}H]-8-MOP to KB cells was time dependent and reached equilibrium in less than 15 min at 4°. Non-specific binding of [^{3}H]-8-MOP was determined in parallel cultures in the presence of an excess of unlabeled 8-MOP (10 μg/mL). Specific binding was calculated by subtracting non-specific binding from total binding.

To assay EGF binding, cells were incubated with 1.5 mL HEPES (25 mM, pH 7.2) buffered DMEM supplemented with 0.1% bovine serum albumin containing ^{125}I-EGF (0.1 μCi/mL, 200 nCi/ng). After 2 hr at 4°, binding assays were stopped as described above and assayed for radioactivity. For Scatchard analysis, cultures were incubated with increasing concentrations of ^{125}I-EGF. Non-specific binding of ^{125}I-EGF was determined in parallel cultures containing an excess of unlabeled EGF (1 μg/mL). Specific binding was calculated as described above. For some experiments, ^{125}I-EGF was assayed after 50 min at 37°.

Treatment of the cells. Cells were pretreated with psoralens and ultraviolet light or TPA to inhibit EGF binding as previously described [5]. Briefly, cells were preincubated with 2 mL of HEPES-buffered Earle's saline containing the indicated concentration of the inhibitors for 30 min. Unless otherwise stated, preincubations were performed at 37°. Psoralen-treated cultures were then pulsed with UVA light emitted from a bank of four BLB fluorescent light tubes (F40 BL, Sylvania) placed approximately 10 cm above the cell culture plates. The incident light on the culture plates was measured with an ultraviolet-radiometer (International Light model IL 442A) and ranged from 3.4 to 6.8 mW/cm^{2}. Cell culture plates were covered with polyacetate sheets which blocked wavelengths below 320 nm. Following drug and light treatments, cells were rinsed with PBS (3×5 mL) and assayed immediately for ligand binding. Drug and/or UVA light treatment, at the doses used in our studies, had no effect on cell viability, as measured by trypan blue dye exclusion, or cell attachment to the culture plates. The treatment protocols also had no effect on [^{3}H]leucine uptake into the KB cells. In each experiment, control plates were protected from UVA light by an opaque cover.

Measurement of internalization and metabolism of cell-associated ^{125}I-EGF. Internalization of ^{125}I-EGF by KB cells was determined by the acetic acid stripping method of Haigler *et al.* [11]. Cells were incubated with 1.5 mL of buffer containing ^{125}I-EGF (0.1 μCi/mL) for appropriate times and then washed with ice-cold PBS (5×5 mL) to remove unbound radiolabel. Two milliliters of ice-cold acidic stripping buffer (0.2 N acetic acid, pH 2.5, 0.5 M NaCl) was then added to each plate. After 6 min, the stripping buffer was removed and duplicate 0.5-mL aliquots of the buffer were taken for counting to determine acid-buffer extractable ^{125}I-EGF. This material represents surface bound ^{125}I-EGF [10]. The cells were then washed with ice-cold PBS (3×5 mL) and solubilized with 2 mL of 1 N NaOH. Duplicate 0.5-mL aliquots of the solubilized cell extracts were counted to determine the amount of ^{125}I-EGF that was internalized. The data are presented as the average of at least two culture plates. In these assays, ^{125}I-EGF binding was assayed at 37° and also at 4°, a temperature which has been shown to inhibit EGF internalization [12].

The metabolism of internalized ^{125}I-EGF was analyzed according to the method of Wiley and Cunningham [13] with some modifications. After pretreatment with TPA or psoralens and UVA light, the cells were incubated at 37° with ^{125}I-EGF in medium to allow the receptor–ligand complex to be internalized and metabolized. After appropriate times the cells were washed with ice-cold PBS (3×5 mL) and the culture plates drained. Cell-associated ^{125}I-EGF, including surface bound material, was extracted by adding 400 μL of ice-cold buffer [20 mM HEPES, pH 7.0, 100 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS), 20 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. Lysed cells were scraped into 1.5-mL Eppendorf microcentrifuge tubes with a rubber policeman. The lysates were centrifuged for 3 min (12,000 *g*) and 100 μL of the supernatants was analyzed for ^{125}I-EGF degradation products using native polyacrylamide gel electrophoresis. The separating gel contained 12.5% polyacrylamide. After electrophoresis, the gel

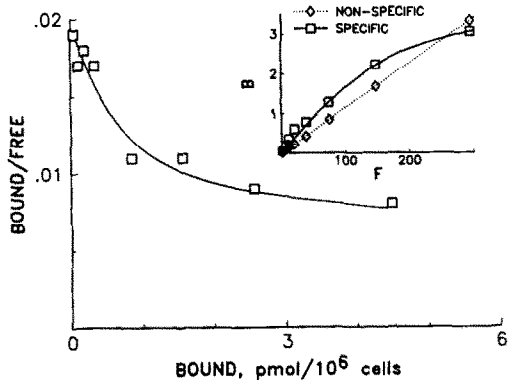

Fig. 1. Scatchard plot of [^{3}H]-8-MOP binding to KB cells. Saturation of specific binding was determined at 4° with increasing concentrations of [^{3}H[-8-MOP.

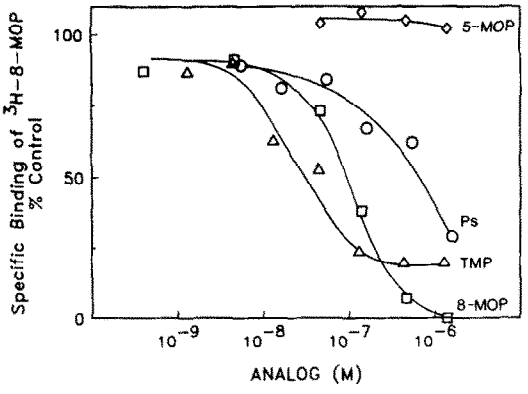

Fig. 2. Competitive inhibition of [^{3}H]-8-MOP binding to KB cells by various psoralen analogs. KB cells were incubated in binding buffer containing [^{3}H]-8-MOP with increasing concentrations of psoralen analogs as described in Materials and Methods. Each data point represents the average of two samples. The ranges were all less than 10%. [^{3}H]-8-MOP control binding was 4010 ± 290 cpm/10^6 cells. Key: (△—△) TMP, (□—□) 8-MOP, (○—○) psoralen, and (◇—◇) 5-MOP.

was cut into 4.5-mm sections and counted for radioactivity.

RESULTS

Characterization of [^{3}H]-8-*MOP binding to KB cells.* We have shown previously that [^{3}H]-8-MOP readily binds to specific receptor sites on a number of cell lines including KB cells [3]. These receptor sites are saturable and a Scatchard analysis of psoralen binding to the cells produced a curvilinear plot (Fig. 1). We interpreted this plot in terms of two independent binding sites [4]. The high-affinity site was estimated to have a dissociation constant (K_d) of 3 nM with 3×10^5 binding sites per cell, while the K_d of the low-affinity site was estimated to be 4.7 μM with 3.9×10^6 binding sites per cell. Several different biologically active psoralen analogs, including TMP, 8-MOP and psoralen, were tested as inhibitors of [^{3}H]-8-MOP binding to KB cells (Fig. 2). TMP was found to be the most effective inhibitor followed by 8-MOP and psoralen.

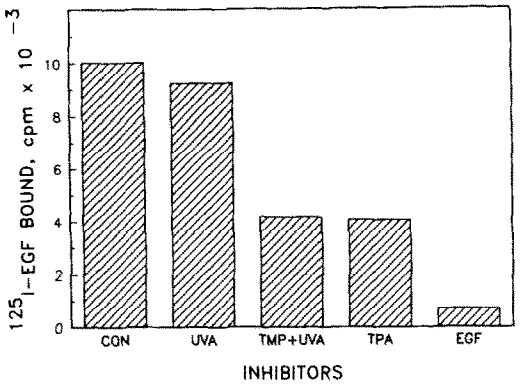

Fig. 3. Inhibition of ^{125}I-EGF binding by TMP and TPA. KB cells were pretreated with buffer, with UVA light (3.7 J/cm^2), TMP (4.4 μM) or TPA (162 nM) at 37° as described in Materials and Methods. After 30 min, cells were assayed for specific ^{125}I-EGF binding at 37°. Each data point represents the average of two samples. The ranges were all less than 9%.

Interestingly, 5-MOP, a biologically active psoralen analog, had no effect on [^{3}H]-8-MOP binding to the cells in the concentration range tested. These data indicate that the psoralen receptor in KB cells can discriminate between structurally related psoralen analogs and that not all active psoralens compete with [^{3}H]-8-MOP for specific binding.

Effect of photoactivated psoralens on ^{125}I-*EGF binding to KB cells.* We have reported previously that TMP and UVA light are potent inhibitors of ^{125}I-EGF binding [5]. To analyze the effects of psoralens and ultraviolet light on ^{125}I-EGF binding to KB cells, cultures were pretreated with TMP and UVA light prior to the binding assays. We found that the combination of TMP (4.4 μM) and UVA light (3.7 J/cm^2) was as effective an inhibitor of ^{125}I-EGF binding to KB cells as the potent tumor promoter TPA (162 nM), a well-recognized inhibitor of EGF binding (Fig. 3). TMP by itself had no effect on ^{125}I-EGF binding (not shown), while UVA light alone produced a slight (6%) inhibition of binding. Under the conditions used, neither TMP and UVA light nor TPA completely inhibited specific binding of ^{125}I-EGF (Fig. 3).

We next examined the effects of temperatures on the ability of TMP and UVA light as well as TPA to inhibit ^{125}I-EGF binding. We found that when the binding assays were performed at either 4° or 37°, ^{125}I-EGF binding was inhibited by excess nonradioactive EGF (Table 1). At 37°, greater specific binding of EGF to the KB cells was observed than at 4° due to growth factor internalization (see further below). When the cells were treated with TMP and UVA light or TPA at 4°, inhibition of EGF binding was decreased when compared to experiments performed at 37°. In further studies, we found that the photoactivated psoralen as well as TPA inhibited ^{125}I-EGF binding at 4° even when cells were pretreated with the drugs at 37° (not shown). These data indicate that inhibition of EGF binding to its receptor by both TPA and TMP/UVA light is temperature dependent.

Table 1. Effect of temperature on the inhibition of ^{125}I-EGF binding

	^{125}I-EGF bound (cpm/10^6 cells)	
	Pretreatment temperature 4°	37°
Control	3,930	10,030
EGF	980 (75)	720 (93)
TPA	3,550 (10)	4,050 (60)
UVA light	3,510 (11)	9,240 (8)
PUVA	3,590 (9)	4,160 (59)

Cultures of KB cells were pretreated with TPA (162 nM) or TMP (4.4 μM) at 4° or 37°. After 30 min, appropriate control and TMP-treated cultures were pulsed with UVA light (3.72 J/cm^2). Cells were then incubated with ^{125}I-EGF at the appropriate temperature as described in Materials and Methods. Unlabeled EGF (1 μg/mL) was added directly to the radiolabeled buffer to determine non-specific binding. The numbers in parentheses represent the percentage inhibition of ^{125}I-EGF binding. Each data point represents the average of two samples. The standard deviations were all less than 9%.

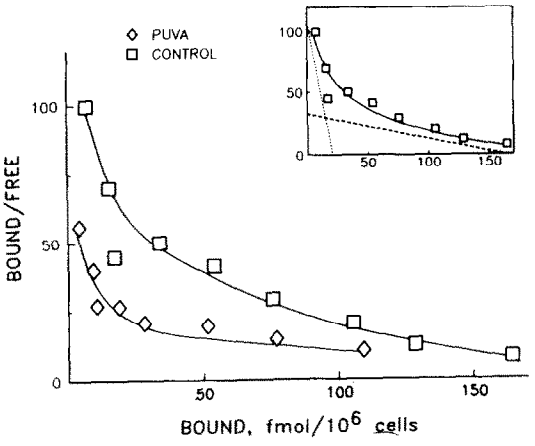

Fig. 5. Scatchard analysis of specific ^{125}I-EGF binding to KB cells. Cells were pretreated with TMP (4.4 μM) and UVA light (3.7 J/cm^2) or with buffer alone before analysis of ^{125}I-EGF binding. The data were resolved into two linear components (see inset). B/F = fmol per 10^6 cells/nM; B = bound, fmol per 10^6 cells.

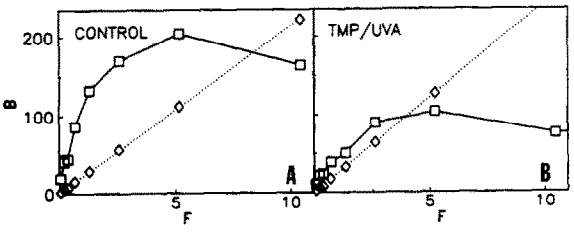

Fig. 4. Effect of PUVA treatment on the saturation of ^{125}I-EGF binding in KB cells. Cultures of KB cells (3×10^6/dish) were incubated with increasing concentrations of ^{125}I-EGF to determine total binding as described in Materials and Methods. In separate cultures, unlabeled EGF (1 μg/mL) was added directly to the radiolabeled binding buffer to quantify non-specific binding. Specific binding was determined by subtracting non-specific binding from total binding. Key: (□—□) specific binding, and (◇—◇) non-specific binding. Panel A, saturation of ^{125}I-EGF binding in control KB cells. Panel B, saturation of ^{125}I-EGF binding in KB cells pretreated with TMP (4.4 μM) and UVA light (3.72 J/cm^2). B = bound, fmol per plate; F = free, nM. Each data point represents the average of two samples. The ranges were all less than 9%.

To study the mechanism underlying the inhibition of EGF binding, KB cells were treated with TMP and UVA light and saturation of EGF binding sites was examined. We found that ^{125}I-EGF binding to KB cells was saturable (Fig. 4). Scatchard analysis of the binding data revealed a curvilinear plot (Fig. 5). This plot was resolved into two classes of binding sites for EGF (Fig. 5, inset): a high-affinity site with a K_d of 0.19 nM and 1.4×10^4 sites per cell and a low-affinity site with a K_d of 5.9 nM and 1.1×10^5 sites per cell (Fig. 5). Treatment of KB cells with photoactivated psoralens decreased the number of high-affinity EGF binding sites to 7.5×10^3 sites per cell while the affinity was largely unchanged (K_d, 0.19 nM, graphic analysis not shown). Similarly, the number of low-affinity binding sites decreased to 7.9×10^4 sites per cell with little change in the binding affinity (K_d, 7.4 nM). Thus, under the conditions used, both the high- and low-affinity receptors for ^{125}I-EGF were affected by TMP and UVA light.

In further studies, we found that psoralen and 8-MOP were less effective in inhibiting ^{125}I-EGF binding to KB cells than TMP. For example, when the cells were treated with psoralen or 8-MOP at a concentration of 1.5 μM and then pulsed with UVA light (3.7 J/cm^2), inhibition was 50% less than that observed with TMP (not shown). Under the same conditions, 5-MOP which had no effect on [^{3}H]-8-MOP binding to the cells (see above) was as effective an inhibitor of EGF binding as 8-MOP (not shown).

Effects of psoralens on internalization of ^{125}I-EGF. Once bound to its receptor, EGF is known to be internalized and metabolized [10]. We found that internalization of EGF by KB cells was temperature dependent occurring to a large extent at 37° but not at 4° (Fig. 6). Therefore, our studies on the effects of psoralens on ^{125}I-EGF internalization were performed at 37°.

We found that surface bound ^{125}I-EGF was rapidly internalized by KB cells. Both surface binding and internalization were inhibited by the combination of TMP and UVA light (Fig. 7). Similar results were observed when the cells were treated with TPA (data not shown). UVA light, which partially inhibited ^{125}I-EGF binding to the cells, also inhibited internalization of ^{125}I-EGF (Fig. 7, right panel). TMP (4.4 μM) alone had no effect on binding or internalization (data not shown). When activated by UVA light, TMP produced a dose-dependent inhibition of ^{125}I-EGF cell surface binding and internalization (Fig. 8). Internalization of ^{125}I-EGF appeared to be more sensitive to the inhibitory effects of TMP than was binding. At the highest

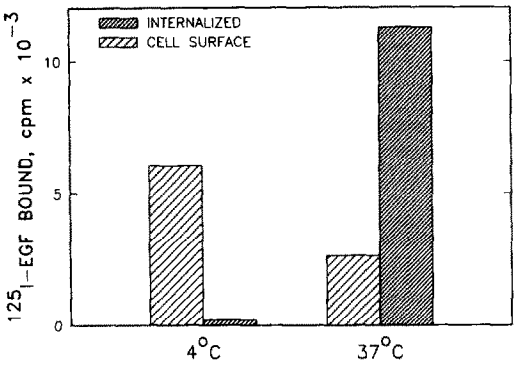

Fig. 6. Effects of temperature on ^{125}I-EGF surface binding and internalization. KB cells were incubated with ^{125}I-EGF at 4° for 2 hr or at 37° for 50 min. Cell surface and internalized ^{125}I-EGF were quantified as described in Materials and Methods. Note that only minimal amounts of ^{125}I-EGF were internalized when the binding assays were performed at 4°. Each data point represents the average of two samples. The ranges were all less than 9%.

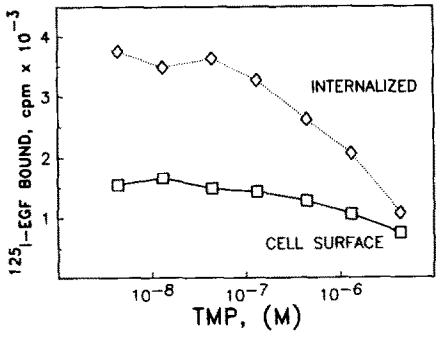

Fig. 8. Effect of PUVA treatment on surface binding and internalization of ^{125}I-EGF. KB cells were incubated at 37° for 30 min in buffer containing increasing concentrations of TMP and then exposed to UVA light (3.7 J/cm^2). ^{125}I-EGF surface binding and internalization were then assayed after 50 min at 37°. Each data point represents the average of two samples. The ranges were all less than 9%.

concentration tested (4.4 μM), inhibition of ^{125}I-EGF binding was 56% while over 75% of the cellular internalization of the ligand was inhibited.

Effect of psoralens on ^{125}I-EGF metabolism. Using native polyacrylamide gel electrophoresis, we found that KB cells rapidly metabolized ^{125}I-EGF. When the radiolabel alone was analyzed, a single peak of activity corresponding to the unmetabolized EGF was visualized (Fig. 9A). Incubation of the cells with ^{125}I-EGF for 5 min at 37° produced two peaks of activity corresponding to unmetabolized ^{125}I-EGF and a faster migrating metabolite (Fig. 9B). After 50 min, ^{125}I-EGF was degraded further (Fig. 9C). Treatment of the cells with UVA light (Fig. 9D) or TMP alone (data not shown) had no effect on the profile of ^{125}I-EGF metabolites. Similarly, the combination of TMP and UVA light, as well as TPA, under conditions which inhibited ^{125}I-EGF binding, also had no effect on degradation of ^{125}I-EGF by the cells (Fig. 9, E and F).

DISCUSSION

In this paper we characterized the binding of psoralens to KB epithelial cells. We demonstrated that, once bound to the cells and photoactivated, these compounds inhibit surface binding and internalization of the peptide growth factor, EGF. The ability of psoralens to modulate the biological activity of specific growth factors in the skin may help to explain the mechanism by which these compounds regulate cell growth and differentiation. Saturation analysis of [^{3}H]-8-MOP binding to KB cells revealed the presence of high-affinity binding sites for the psoralens. Similar results were reported previously using human HeLa cells [3]. However, lower concentrations of 8-MOP were required to saturate high-affinity binding sites in KB cells than in HeLa cells (K_d of 19 nM). HeLa is a cell line derived from a cervical carcinoma, whereas KB cells are derived from an oral carcinoma. Differences in the kinetics of [^{3}H]-8-MOP binding to HeLa and KB cells may be due to their distinct tissue origins.

In competitive binding assays we found that psoralen and several biologically active analogs including TMP and 8-MOP were effective inhibitors

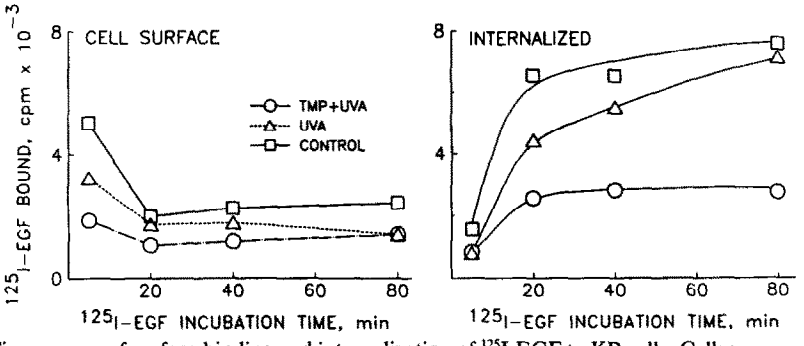

Fig. 7. Time course of surface binding and internalization of ^{125}I-EGF to KB cells. Cells were pretreated with UVA light (3.7 J/cm^2) alone or TMP (4.4 μM) followed by UVA light and then incubated at 37° in ^{125}I-EGF binding buffer for increasing periods of time. At the indicated time points, acid-sensitive (cell surface) and acid-insensitive (internalized) radioactivity was determined. Each data point represents the average of two samples. The ranges were all less than 9%.

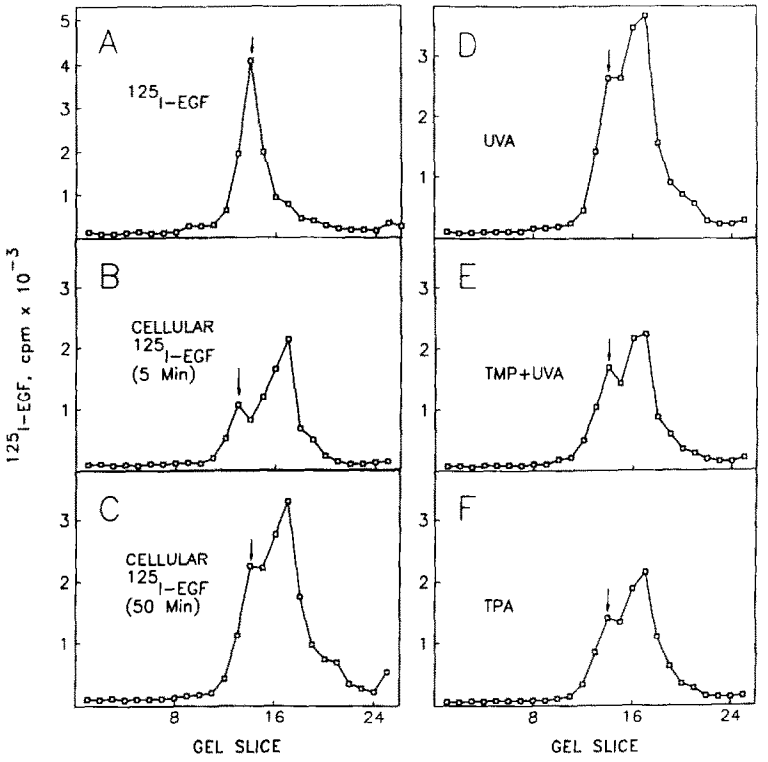

Fig. 9. Cellular degradation of ^{125}I-EGF in KB cells. Cells were pretreated with buffer, UVA light (3.7 J/cm^2) alone, TMP (4.4 μM) and UVA light or TPA (16.2 nM) and then incubated with ^{125}I-EGF at 37°. ^{125}I-EGF and its degradation products were extracted and separated in 12.5% polyacrylamide gels as described in Materials and Methods. Gels were cut into 4.5-mm sections and counted for radioactivity. Panel A, undegraded ^{125}I-EGF (indicated by the arrow); panel B, ^{125}I-EGF metabolites after 5 min; panel C, ^{125}I-EGF after 50 min; panel D, ^{125}I-EGF metabolites 50 min after UVA light treatment; panel E, ^{125}I-EGF metabolites 50 min after TMP and UVA light treatment; and panel F, ^{125}I-EGF metabolites 50 min after TPA treatment.

of [^{3}H]-8-MOP binding to KB cells. TMP, the most lipophilic of the psoralen analogs tested, was the most effective inhibitor of [^{3}H]-8-MOP binding. For reasons that are not readily apparent, 5-MOP, in the concentration range tested, had no effect on [^{3}H]-8-MOP binding to the cells despite the fact that it is an active skin photosensitizer [14] and that it inhibited ^{125}I-EGF binding to KB cells. It is possible that higher concentrations of 5-MOP are required to inhibit 8-MOP receptor binding. Alternatively, KB cells may possess receptors for 5-MOP that are distinct from 8-MOP receptors. In separate studies, 5-MOP was found to inhibit [^{3}H]-8-MOP binding to B16/C3 cells, a melanocyte-derived cell line (unpublished studies). Thus, the ability of 5-MOP to inhibit [^{3}H]-8-MOP binding may be cell-type specific. Differences in the binding properties of 8-MOP and 5-MOP to various cell types in the skin may underlie the distinct clinical properties of these two drugs [14].

One of the most rapid biological effects resulting from PUVA treatment is the inhibition of EGF binding [5]. This inhibition occurs in a manner similar to that observed with the phorbol ester, TPA [5, 6, 15]. Both photoactivated psoralens and TPA modulate the growth of epidermal cells and PUVA, like TPA, may be a tumor promoter [16]. It is possible that the interaction of these two compounds with the EGF receptor is a common mechanism underlying their actions in the skin. Scatchard analysis of binding of ^{125}I-EGF to KB cells revealed both high- and low-affinity receptors for EGF. PUVA treatment decreased the number of both receptor populations. We previously reported that, in the mouse keratinocyte cell line PAM 212, which also possesses high- and low-affinity EGF receptors, PUVA treatment selectively decreases the number of high-affinity receptors [5]. As noted above for psoralen binding, these differences in inhibition of EGF binding may be related to the different tissue origin of the cells.

Of particular interest was our observation that modulation of EGF binding by TMP and UVA light, as well by TPA, was temperature dependent. Similar results with TPA have been reported previously using mouse fibroblasts, mink lung cells [15] and human HeLa cells [6]. Taken together, these data suggest that cellular metabolic activity is required for the photoactivated psoralens and TPA to inhibit EGF binding. However, at this time, we cannot exclude the possibility that temperature sensitivity to EGF binding inhibition was due to differences in the kinetics of either psoralen or TPA binding at 4° and 37°.

From our data it is apparent that PUVA treatment inhibited not only EGF cell surface receptor binding, but also internalization of the EGF–receptor complex. Similar findings have been reported previously with TPA in pancreatic acinar cells [17]. In our studies, internalization of ^{125}I-EGF appeared to be more sensitive to the inhibitory effects of PUVA than was cell surface binding. Internalization of the EGF–receptor complex is a temperature-dependent process and is known to occur via the clathrin-coated pit pathway [18, 19]. It has been suggested that the internalization process may play a role in the transduction of the signal from the growth factor to the nucleus [20]. Thus, the ability of the photoactivated psoralens to interfere with EGF internalization may contribute to the mechanism by which these compounds regulate growth.

Once ligands are internalized in cells by receptor-mediated endocytosis, degradation may occur [18]. Altered ligand degradation following drug treatment may be indicative of abnormal cellular metabolism. Haigler *et al.* [10] have shown that resistance to diphtheria toxin is associated with altered EGF degradation and suggested that this was due to a defect in the intracellular processing of EGF. In our studies, we found that KB cells rapidly metabolize ^{125}I-EGF into discrete forms with altered electrophoretic mobility. Furthermore, treatment of the cells with either PUVA or TPA, although decreasing the amount of internalized radiolabel, appeared to have no effect on ^{125}I-EGF metabolism. Thus, in KB cells, the activity of these EGF binding inhibitors may be limited to the cell surface membrane.

The EGF receptor is a tyrosine-specific protein kinase whose activity is augmented following EGF binding [7, 21]. TPA inhibits EGF binding as well as EGF-stimulated receptor kinase activity and this is thought to mediate, at least in part, its biological actions [22, 23]. It is possible that the EGF receptor kinase is also the target for photoactivated psoralens. However, TPA and psoralens appear to act by distinct mechanisms. These compounds do not directly compete with one another in cellular binding assays [3]. In addition, TPA is known to bind to and activate protein kinase C [24]. We found that photoactivated psoralens do not directly activate protein kinase C (manuscript in preparation). It may be that photoactivated psoralens activate protein kinase C by an indirect mechanism, possibly by inducing the synthesis of diacylglycerol, an endogenous protein kinase C activator [24]. Further studies are required to examine this possibility and to explore other mechanisms by which the psoralens interact with the EGF receptor to modulate cell growth.

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