Ultraviolet Radiation Alters Choline Phospholipid Metabolism in Human Keratinocytes

Vincent DeLeo, M.D., Sara Scheide, M.S., Joel Meshulam, M.D., Debra Hanson, B.S., and Alice Cardullo, M.D.
Department of Dermatology, Columbia University, New York, New York, U.S.A.

Ultraviolet radiation B (UVB – 290 – 320 nm) induces inflammation and hyperproliferation in human epidermis. This response is associated with the recovery from irradiated skin of inflammatory mediators derived from membrane phospholipids. We have previously reported that UVB stimulates the production of such mediators by human keratinocytes (HK) in culture. In these studies we examined the effect of UVB on the metabolism of choline containing phospholipids in HK prelabeled with [^{3}H] choline.

UVB (400 – 1600J/m^2) stimulated a dose dependent release of [3H] choline from HK within minutes of irradiation. Examination of media extracts by paper chromatography revealed that the released [^{3}H] choline was predominately in the form of glycerophosphorylcholine. Examination of label remaining in membranes of cells after irradiation by acid precipitation and HPLC revealed that the origin of the released [^{3}H] choline was the membrane phosphatidylcholine/lysophosphatidylcholine. These data support a concept of UVB stimulation of both a phospholipase A (1 or 2) and a lysophospholipase. These UVB induced alterations of HK membrane phospholipid metabolism likely have profound effects on UVB-induced inflammation and control of cell growth in human skin. *J Invest Dermatol 91: 303 – 308, 1988*

Solar ultraviolet radiation (UV) is a ubiquitous environmental agent known to produce numerous effects in human skin, including inflammation, pigmentation, alteration of immune function, and carcinogenesis [1]. The mechanism by which such radiation evokes most cutaneous photobiologic responses remains poorly defined. Relatively little attention has been paid to the possible role of membrane constituents in the mediation of UV-induced skin changes [2]. This is in spite of evidence from epidemiologic, in vivo, and in vitro studies, which suggests that UV damage in skin results in stimulation of many biologic processes analogous to those induced by membrane active agents like chemical tumor promoters and polypeptide growth factors [3 – 7]. Such agents are known to act through alterations of membrane phospholipid structure and function, including phospholipase activation, second messenger induction, and lipid derived arachidonic acid metabolite production [8].

We have utilized mammalian cells in culture to investigate the effect of UV on membrane related phenomena. We have previously reported UV induced release of arachidonic acid from membrane phospholipids in murine fibroblasts [9] and human epidermal keratinocytes [6] as well as UV-induced stimulation of prostaglandin production.

The choline containing phospholipids (phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin) comprise a major component of the lipid structure of membranes of human skin cells [10,11]. To further investigate UV-membrane interaction we examined the effect of UVB (290 – 320 nm) on choline metabolism of human epidermal keratinocytes in culture.

Manuscript received October 20, 1987; accepted for publication April 6, 1988.

This work was supported by NIH Grant #AR33663.

Reprint Requests: Vincent DeLeo, M.D., Department of Dermatology, Columbia University, 630 West 168th Street, New York, New York 10032.

Abbreviations:
AA: Arachidonic Acid
Ch: Choline
HK: Human keratinocytes
UV: Ultraviolet radiation
UVB: Ultraviolet B radiation (290 – 320 nm)

MATERIALS AND METHODS

Epidermal Cell Cultures Cells were grown according to the method of Eisinger et al [12] described in detail previously. In brief, normal human breast skin was obtained from surgical specimens. The skin was cut into small discs and exposed for a 12 – 14-h period to a 0.25% trypsin solution. The tryptic activity was stopped with fetal calf serum 20%. The epidermis from each disc was peeled from the dermis and the epidermal discs were pooled. A single cell suspension of epidermal cells was produced by a vigorous trituration in trypsin : EDTA solution. The trypsin : EDTA solution was inactivated with serum. Cells were centrifuged, the trypsin : EDTA was removed, and the cells were counted. After dilution with keratinocyte medium (KM + 10) the cells were seeded at appropriate densities in 35 mm or 60 mm dishes (Corning).

Media, Solutions, and Chemicals EDTA and hydrocortisone were obtained from Sigma (St. Louis, MO); trypsin (1:250) was obtained from Gibco (Grand Island, NY); and L-glutamine, antibiotic-antimycotic solution and non-essential amino acid mixture were obtained from Gibco.

Keratinocyte medium (KM) consisted of minimal essential medium with Earle's salts (Gibco) plus nonessential amino acids (0.1 nM), 2 nm L-glutamine, hydrocortisone (0.4 ug/ml), penicillin (100/U/ml), streptomycin (.1 mg/ml), and fungizone (2.5 ug/ml). Seeding and growth medium included 10% heat-inactivated fetal calf serum (KM + 10). Labeling medum was KM + 10 without hydrocortisone, and assay medium contained no serum (KM + 0) or hydrocortisone. Hanks' buffered salt solution without phenol red (HBSS) was obtained for Gibco. Methyl-^{3}H-Choline, 1 uCi/ml; 77 Ci/mmol was obtained from Amersham International (United Kingdom). Choline, phosphorylcholine, glycerophosphorylcho-

0022-202X/88/$03.50

line, phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin were obtained from Sigma.

Light Source The light source utilized in these experiments consisted of a bank of 6 Philips TL20W/12 fluorescent tubes. The irradiance was 12 W/m² as measured by an International Light Research Radiometer, IL500A with a UVB probe SEE1240 (275–303 nm). The special emission of the source was 270–360 nm with emission in the UVB range (290–320 nm) accounting for 60% of the irradiance.

Release of Label Assay Human keratinocytes (passage 0) were seeded into dishes at a constant density ($0.9-1.3 \times 10^5/cm^2$) in KM + 10. Cells for each experiment were obtained from a single primary culture and were used at densities of approximately 6×10^6 cells and 2×10^6 cells per dish for 60 and 35 mm dishes, respectively. Media were changed every 3–4 d and the cells were allowed to grow until 90% confluency was achieved (9–14 d). The cells were prelabeled with [³H]choline ([³H]Ch) in labeling medium (2 uCi/ml) for a 24 h period under routine incubation conditions. After the labeling period, the media were removed and the cells were washed three times with HBSS without calcium. A small amount of HBSS with calcium was then added to each of the dishes to prevent dessication during irradiation. The cells were irradiated with selected doses of UVB. Cells treated in the same fashion were placed in the field of irradiation and covered with opaque foil to serve as sham irradiated controls. Immediately after irradiation, assay medium was added to the dishes and the cells were incubated for selected periods of time. The media were removed, centrifuged at 10,000 RPM to remove detached cells, and an aliquot was assayed for radioactivity by liquid scintillation spectroscopy. The data calculated in terms of cpm/dish represent the total release of label and consist of all labeled choline metabolites. The data points are the means of duplicate or triplicate dishes (SEM ≤ 10%). The data, in some cases, are presented in terms of *% Control:*

$$\% \text{ Control} = \frac{\overline{X} \text{ cpm/dish (Irridated)}}{\overline{X} \text{ cpm/dish (Control)}} \times 100$$

Identification of Specific Choline Metabolites Cells were grown, prelabeled, and irradiated or sham treated as outlined above. At selected times, media were removed from irradiated and control cells. Trichloroacetic acid (TCA) was added to the media to give a final concentration of 10% and the mixture was vortexed. The TCA/media was centrifuged at 1000 RPM for 20 min to precipitate macromolecules. The supernatant was then mixed with two volumes of ethyl ether, centrifuged, and the non-polar layer was discarded. The extraction was repeated and the pooled water layer was freeze dried and stored at −70°C until utilized in the following paper chromatographic system [13,14].

The freeze dried samples from control and irradiated cells were re-solubilized in a small quantity of water and spotted onto Whatmann 3 mm papers (20 × 24 m) with known standards for water soluble choline metabolites. The chromatographs were developed in a solution of ammonium acetate buffer (1 M; pH-5.0); 95% ETOH, [30:70]. The chromatographs were developed for 18 h and then air dried and standards were visualized with iodine vapor. Sample lanes were cut into 1-cm pieces and the radioactivity in each piece was determined by liquid scintillation counting. (Each paper was placed in the scintillation vial with 1.4 ml of water and placed on a shaker at 37°C for 1 h, before addition of cocktail). The identity of the radiolabeled choline metabolites was determined by co-migration of radiolabel with known standards.

Origin of Released Choline Metabolites In order to determine the origin of the [³H]Ch released into media by irradiation two different types of experiments were performed. In the first type the acid soluble and acid precipitable pools of radioactivity were determined in sham treated and irradiated cultures by a modification of techniques described by Plagemann [15]. In the second type of experiment the [³H]Ch-labeled membrane phosholipids of cells, irradiated and control, were extracted and separated by HPLC.

Acid Soluble/Acid Preciptable Pools Cells were prelabeled with [³H]Ch and irradiated or sham treated as outlined above. After incubation the media were removed and an aliquot was assayed for released label. The cells were then washed quickly with H_2O at 4°C. The cells were incubated with cold trichloroacetic acid (TCA) 10% at 4°C for 30 min. The TCA was removed and the cells were washed twice with cold TCA. The TCA from the incubation and the two washes were pooled and an aliquot was assayed by liquid scintillation to determine the acid soluble pool. This value represented the intracellular pool of [³H] choline metabolites. The cells were then solubilized in SDS 2% and an aliquot was assayed by liquid scintillation to determine the acid precipitable pool or the membrane pool of [³H] choline.

Identification of [³H] Labeled Cellular Phospholipids In order to determine the specific [³H]Ch membrane components altered by UVB, cells were prelabeled with [³H]Ch and irradiated or sham treated as outlined. At appropriate times after treatment the media were removed from irradiated and control cells. The cells were washed × 2 with HBSS and cellular lipids were extracted utilizing a modified Folch procedure [16]. Ice cold methanol was added to the dishes and the cells were then scraped into conical tubes. Cold chloroform was added to each tube for a chloroform:methanol ratio of 2:1. The cells were ultrasonicated for 3 min on ice and centrifuged (400 g × 1 min at −4°). The supernatant was removed to another tube and water was added to give a chloroform:methanol:water ratio of 8:4:3. The solution was mixed and centrifuged. The chloroform layer was removed and dried under N_2. The dried lipid fraction was stored in a small quantity of chloroform at −70° until examined in the HPLC system listed below.

The separations were carried out utilizing a Perkin Elmer Model HPLC equipped with a Model 55 Variable Wavelength UV-Vis spectrophotometer, vertical pen recorder, and automatic fraction collector. The solvent program was an isocratic elution of acetonitrile:methanol:sulfuric acid, 100:3:0.05 [17], and the flow rate was set at 1.0 ml/min. The samples were injected with standards for phosphatidylcholine, lysophosphatidylcholine, platelet activating factor, and sphingomyelin. The standard positions were identified by UV absorption at 201 nm. The eluate was collected in 1-min fractions. The radiolable eluting in each fraction was assayed by liquid scintillation and the identity of [³H] labeled choline containing phospholipids was determined by co-elution of radiolable with standards.

RESULTS

Human keratinocytes prelabeled with [³H] choline for a 24-h period routinely incorporated 40–60% of the label from the medium. When prelabeled control cells were washed and incubated for a 2–4-h period in assay buffer, radioactivity released into the medium varied among experiments but was in the range of 1.5 to 3% of the total incorporated. Figure 1 details the results of a typical dose response experiment in which the cells were irradiated or sham treated with UVB in doses from 400 to 2400 J/m² and then incubated for a 4-h period before removal of media for assay. UVB stimulated the release of [³H]Ch in a dose-dependent fashion between 400 and 1600 J/m². The response plateaued between the 1600 and 2400 J/m² doses. This experiment was repeated on numerous occasions with similar results. The 1600 J/m² dose routinely resulted in release of label of 2 to 4 times the control release or 6.3 ± 0.5 ($\overline{X} \pm$ SEM%; N = 4) of the total incorporated radioactivity after a 4 h incubation.

Figure 2 gives the results of a time course experiment. Cells were prelabeled and irradiated or sham treated with 1600 J/m² UVB. The release of label was determined at various times post-irradiation, from immediately up to 24 h. The UVB-induced release began within the first 15 min post-irradiation (180% control) and reached levels of 400% control at 4 h. The increase continued and at the 24-h timepoint the radiolabel released by irradiated cells was 10X that released by control cells.

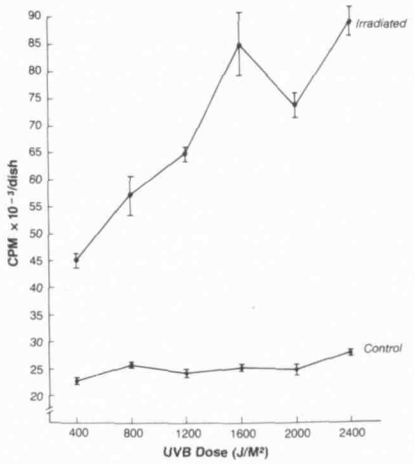

Figure 1. The effect of UVB on release of label from human keratinocytes. Human keratinocytes were prelabeled with [^{3}H] Choline and irradiated or sham treated with doses indicated. Cells were incubated for 4 h before assay. Data represent X ± SEM of triplicate determinations.

The data for the UVB-induced release of [^{3}H]Ch as measured in the above release of label studies are a measure of all [^{3}H]Ch metabolites released into the medium. Because various metabolites containing [^{3}H]Ch could be hydrolyzed from membrane phospholipids by the activity of different lipases (see Fig 4) we were interested more specifically in investigating the mechanism of [^{3}H]Ch release by quantitating the UVB-induced alteration of release of specific [^{3}H]Ch metabolites. In these studies cells were prelabeled and then irradiated with 1800 J/m² (or sham treated). After a 4-h incubation the water soluble choline metabolites from the media of irradiated and sham treated cells were extracted and radiolabeled metabolites were identified by co-migration with known standards in a paper chromatographic system. The results of a representative experiment are shown in Fig 3. Although there was some increase in recovery of radiolabel co-migrating with phosphorylcholine and choline standards, the overwhelming increase occured in fractions co-migrating with the standard glycerophosphorylcholine. It should be noted that there was no radiolabel co-migrating with the standard for phosphorylcholine in extracts from control cells in this experiment. In other experiments phosphorylcholine routinely represents less than 3% of the total radiolabel in media extracts from control cells. This is to be expected because no phosphatidylcholine specify phospholipase C has been identified in human epidermis.

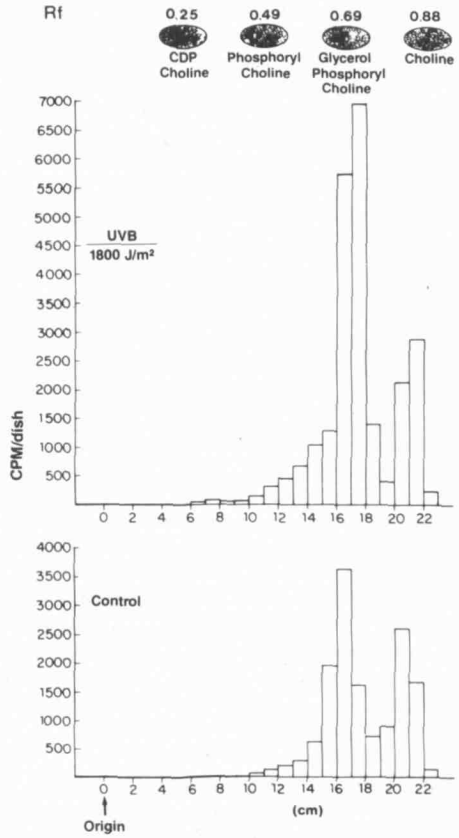

Figure 3. Identification of specific [^{3}H] choline metabolites released by UVB treatment. Human keratinocytes were prelabeled with [^{3}H] choline and then irradiated or sham treated with UVB, 1800 J/m². Cells were incubated for 4 h. Water soluble metabolites were extracted and examined by paper chromatography as outlined in *Methods*.

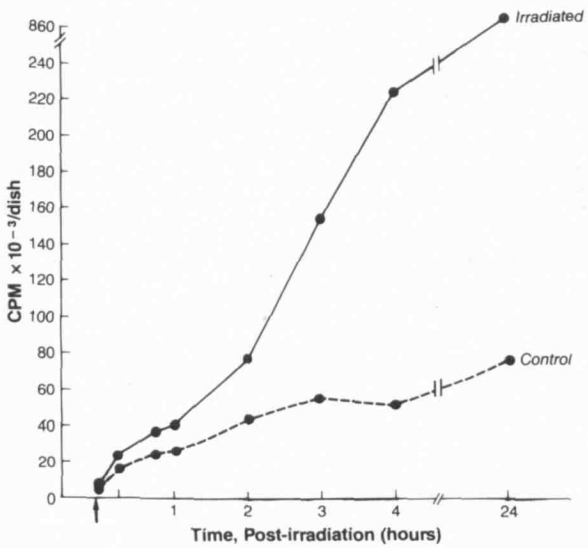

Figure 2. The time course of UVB-induced release of label from human keratinocytes. Human keratinocytes were prelabeled with [^{3}H] choline and then irradiated or sham treated with 1600 J/m² UVB. Media were removed and assayed for release of label at selected times post-irradiation. Data represent X of triplicate observations (SEM ≤ 10%).

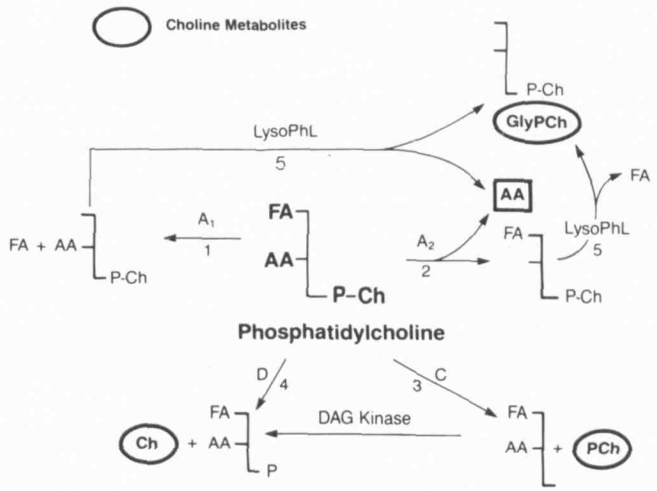

Figure 4. Phosphatidylcholine metabolism. [P-Ch — phosphorylcholine; CH — Choline; GlyPch — glycerophosphorylcholine; FA — saturated fatty acid; AA — arachidonic acid; PAF — platelet activating factor; DAG — diacylglyceride; Lysophyl — lysophospholipase.]

Table I. UVB Induced Release of [^{3}H] Choline from Prelabelled Human Keratinocytes[a]

	Radioactivity – cpm × 10^3 (% of Total)	
	Control	UVB
Medium	41.3 ± 1.7 (1.8)	85.7 ± 1.5 (3.6)
Intracellular Pool	197.2 ± 8.2 (8.6)	254.1 ± 2.6 (10.6)
Membrane Pool	2,067.0 ± 28.2 (89.6)	2,057.0 ± 18.3 (85.8)
TOTAL	2,305.5 (100)	2,396.8 (100)

[a] Human Keratinocytes were prelabeled and irradiated or sham treated with 1600 J/m^2 UVB. Cells were incubated for 4 h before extraction and assay of various fractions as described in "Methods." Data are X CPM ± SEM for triplicate dishes.

[^{3}H] Choline in mammalian cells is incorporated into membrane phospholipids *(membrane pool)* but can also exist as water soluble choline metabolites in an intracellular pool. In order to determine the origin of the UVB-induced [^{3}H]ch released we examined the effect of UVB on the two cellular pools by a standard cold acid precipitation technique. The results of that experiment are shown in Table I. After UVB (1600 J/m^2) or sham treatment radiolabel was assayed in the medium, the *intracellular pool* (TCA soluble), and *membrane pool* (TCA precipitable-phosopholipids). When the cells were incubated for 4 h after sham treatment 1.8%, 8.6%, and 89.6% of the label was associated with the medium, intracellular, and membrane pools, respectively. After irradiation the values were 3.6%, 10.6%, and 85.6%, respectively. The value for the membrane associated radioactivity decreased after irradiation, while the radioactivity in the medium and the intracellular pool increased, suggesting that the UVB radiation resulted in release of label from the membrane pool into the medium and intracellular pools. There was no indication of a simple increase in diffusion from the intra-cellular into the extra-cellular space as might be expected if UVB induced a simple increase in membrane permeability.

We also examined the effect of UVB on membrane phospholipids by examining the effect of radiation on remaining label in specific phospholipids in the keratinocytes. In these experiments the cells were prelabeled and irradiated or sham treated with 2000 J/m^2 UVB. After a 3-h incubation the media were removed and the cellular phospholipids were extracted and separated by HPLC as outlined. The quantity of [^{3}H]ch-labeled phosopholipids as identified by co-elution with standards for sham treated and irradiated cells are given in Table II. Because phosphatidylcholine and lysophosphatidylcholine separated poorly in the HPLC system utilized, the radiolabel co-eluting with these two standards is combined. In control cells 82% of the label was associated with the phosphatidylcholine/lysophosphatidylcholine fraction and 15% eluted in the sphingomyelin fraction. (Nearly 98% of the extracted radioactivity eluted in those two fractions.) No significant radiolabel co-eluted with the other known choline containing phospholipid, platelet activating factor. When cells were irradiated, the label in both fractions decreased, but the largest decrease was from the phosphatidylcholine/lysophosphatidylcholine component, suggesting that the UVB-induced release of [^{3}H]Ch was predominantly from that phosopholipid class.

DISCUSSION

When solar ultraviolet radiation (UV) interacts with human skin a complex process ensues which includes acutely, inflammation, hyperproliferation (sun burn), pigmentation (tanning), and chronically (if the cumulative dose has been sufficient) actinic cancer production and photo-aging. The mechanisms by which UV induces these responses is poorly understood [1]. The photochemistry and photobiologic consequences of UV-DNA interaction have been extensively studied. UV-induced DNA lesions are thought to be the initiators of actinic skin cancers, at least non-melanoma tumors. The relationship of these lesions to the acute changes of inflammation, hyperproliferation, and pigmentation have not been delineated [2].

The carcinogenic process in mouse skin has classically been divided into two stages: *initiation,* involving a DNA altering carcinogen and *promotion,* involving agents like the phorbol ester promoters that act at the cell membrane level. The effect of these latter agents on animal and human skin includes inflammation and hyperproliferation. All three effects, inflammation, proliferation, and tumor promotion, have been shown to be mediated by membrane derived second messenger systems involving alterations of membrane phospholipids [8].

Ultraviolet radiation acts as a complete carcinogen in animal skin. It may be that such radiation acts not only as an initator but also as a promotor, especially because, like chemical tumor promoters, UV induces inflammation and hyperproliferation in the skin [3]. This raises the possibility that UV may effect changes in membrane phospholipid metabolism analagous to those changes induced by the phorbol esters.

Basic understanding of the role of phospholipid metabolism in the structure and function of human skin has increased greatly since the initial report of phospholipid quantitation in desquamated skin by Eckstein and Wile in 1926 [18]. As early as 1932 Koonman observed that a striking decrease in phospholipid content occured as cells of human epidermis differentiated and became keratinized [19]. In fact, the process of membrane phospholipid transformaton into neutral lipids by the activity of epidermal lipases results in one of the most significant functions of mammalian skin; the production of an interface with the environment maintained by a keratinized permeability barrier [20–22]. Phospholipids constitute the major lipid component of the membrane of undifferentiated epidermal cells of pig and human skin (but not rat skin) [10,11,23]; this includes particularly large quantities of the choline containing phospholipids, phosphatidylcholine, and sphingomyelin.

In addition to the importance of phospholipids in the structure and differentiation of the epidermis and their involvement in second messenger generation, phospholipids are also the origin of two important groups of membrane derived mediators. The first group are the arachidonate metabolites: the prostaglandins, leukotrienes, and monohydroxyeicosatetraenoic acids (HETE). The second group are the phosphatidylcholine metabolites, platelet activating

Table II. The Effect of UVB on Membrane [^{3}H] Choline Labeled Phospholipids Separated by HPLC[a]

	Radiolabel – cpm × 10^3		
	Phosphatidylcholine & Lysophosphatidylcholine	Sphingomeylin	Total Extracted
Control	1,988.8	374.5	2,418.8
UVB	1,885.5	359.0	2,299.9
Difference	103.3	15.4	120.9
(% Total)	(85.4)	(12.7)	(100)

[a] Human keratinocytes were prelabeled with [^{3}H] choline and then irradiated with 2000 J/m^2 UVB. The cells were incubated for 3 h and then extracted and extracts were examined by HPLC as described in "Methods."

factor [24,25], and lysolecithin [26]. These agents are capable of producing edema, erythema, and leukocyte chemotaxis in human and animal skin [27–34] as well as alterations of epidermal keratinocyte growth [35–38]. In addition, these mediators may regulate the activity of epidermal melanocytes [39] and Langerhans cells [40].

We have previously shown that UVB induced the release of arachidonic acid from the membrane phospholipids of cells, including human keratinocytes, in culture and that UVB stimulated cells in culture to synthesize arachidonic acid metabolites [6,9]. In this communication we present data that show that UV also induces the release of another membrane phospholipid component, choline, from prelabeled human keratinocytes in culture. This response occurs in a dose dependent fashion and in the same physiologic dose range necessary to induce AA release in this cell type. Furthermore the time course for release of these two phospholipid components is similar, beginning immediately after irradiation and continuing through at least 24 h. This suggests a related mechanism, as one might expect, because both choline and arachidonic acid are components of the phospholipid, phosphatidylcholine (Fig 4).

Utilization of the system of cells prelabeled with [^{3}H]Ch as described in this communication combined with chromatographic identification of specific water soluable [^{3}H]Ch metabolites released by a stimulus can serve as an indirect method of identifying phospholipase activation in intact cells. As detailed in Fig 4, if an agent induces release of phosphorylcholine, the mechanism involved in phospholipase C activation. Similarly, induction of release of choline suggests phospholipase D activation. In fact, using this system we have found that commercially available (Sigma) phospholipase C induced phosphatidylcholine release and brown recluse spider venom (phospholipase D) induced choline release (unpublished observation).

In studies presented in this communication UVB induced release of glycerophosphorylcholine. This implicates a dual enzyme activation: a phospholipase A (either 1 or 2) combined with a lysophospholipase. Either combination would result in release of AA and glycerophosphorylcholine from membrane phosphatidylcholine. It should be noted that lysophospholipase activation is suggested, not proven, by this indirect method. In addition, increased lysophospholipase activity, if present, could be due to increased availability of substrate rather than stimulation. This mechanism of UVB action is supported by our previous observation of UVB-induced AA release [6] and by the observation of Ziboh et al [41], who found UVB stimulation of phospholipase A_2 in enzyme preparations from guinea pig skin. Additionally, both phospholipase A_2 and lysophospholipase activities have been identified in human epidermis [42–44].

To further document this UV-induced phospholipid alteration we have shown that the UVB-stimulated release of glycerophosphorylcholine originated from membrane phospholipids, specifically the phosphatidylcholine fraction of the keratinocyte membrane.

Our studies revealed some UV-induced alteration of the other major choline containing membrane constituent, sphingomyelin. The activity of various sphigomyelinases is less well defined than the lipases outlined in Fig 4 so that the mechanism of UV degradation of this lipid is less easily hypothesized. A sphingomyelinase specific phospholipase C has been identified, however, in human skin preparations [42].

The data presented here and in our previous reports [6,9] suggest that UVB stimulates phospholipase activation and membrane phospholipid degradation. Finally, these AA metabolites as well as lipase activation may act through second messenger systems to alter control of cell growth. Such alteration may lead to proliferation and promotion of skin tumor production.

Finally, the rapidity of the responses described in this manuscript suggests that in addition to its well documented effect on DNA, UVB also has profound, direct effects on membranes of cells of human skin.

REFERENCES

1. Parrish JA: Response of skin to viable and ultraviolet radiation. In: Goldsmith LA (ed.), Biochemistry and Physiology of the Skin. Oxford Union Press, New York, 1983
2. Smith KC: Photobiology and photomedicine: The future is bright. J Invest Dermatol 77:2–7, 1981
3. Rundel RD: Promotional effects of ultraviolet radiation on human basal and squamous cell carcinoma. Photochem Photobiol 34:569–575, 1983
4. Gilchrest BA, Soter NA, Stoff JS, Hihm MC: The human sunburn reaction: histologic and biochemical studies. J Am Acad Dermatol 5:411–422, 1981
5. Ziboh VA, Lord JT, Uematsu S, Blick G: Activation of phospholipase A_2 and increased release of prostaglandin precursor from skin by ultraviolet irradiation. J Invest Dermatol 70:211, 1979
6. DeLeo VA, Horlick H, Hanson D, Eisinger M, Harber LC: Ultraviolet radiation induces changes in membrane metabolism of human keratinocytes in culture. J Invest Dermatol 83:323–326, 1984
7. Laskin JD, Lee E, Laskin DL, Gallo MA: Psoralens potentiate ultraviolet light-induced inhibition of epidermal growth factor binding. Proc Natl Acad Sci USA Cell Biology 83:8211–8215, 1986
8. Rozengurt E: Early signals in the mitogenic response. Science 234:161–166, 1986
9. DeLeo VA, Hanson D, Weinstein IB, Harber LC: Ultraviolet radiation stimulates the release of arachidonic acid from mammalian cells in culture. Photchm Photobiol 41:51–56, 1985
10. Gray GM, Yardley HJ: Lipid composition of cells isolated from pig, human and rat epidermis. J Lipid Res 16:434–440, 1975
11. Tsambaos D, Kalofoutis Am Stratigos J, Miras C, Capentanakis J: Thin-layer chromatography of phospholipid components of normal and psoriatic epidermis. Br J Dermatol 97:135–138, 1977
12. Eisinger M, Lee JS, Hefton JM, Drarzuynkiewica JW, DeHaarven E: Human epidermal cell cultures: Growth and differentiation in the absence of dermal components and medium supplements. Prc Natl Acad Sci USA 76:5340–5344, 1979
13. Infante JP, Kinsella JE: Phospholipid synthesis in mammary tissue; Choline and ethanolamine kinases: kinetic evidence for two discrete active sites. Lipids 10:727–735, 1971
14. Plagemann PGW: Choline metabolism and membrane formation in rat hepatoma cells grown in suspension culture. J Cell Biology 42:766–782, 1969
15. Plagemann PGW: Choline metabolism and membrane formation in rat hepatoma cells grown in suspension culture. I. Incorporation of choline into phosphatidylcholine of mitochondria and other membranous structures and effect of metabolic inhibitors. Arch Bhichem Biophys 128:70, 1968
16. Folch JM, Lees GH, Stanley S: J Biol Chem 226:497–450, 1957
17. Kaduce TGL, Nortoer KC, Spector AA: A rapid isocratic method for phospholipid separation by high performance liquid chromatography. J Lipid Res 24:1398–1403, 1983
18. Eckstein HC, Wile UJ: Phospholipid content of the cutaneous epithelium of man. J Biol Chem 69:181–188, 1926
19. Koonman DJ: Lipids of the skin. Arch Dermatol 25:444–450, 1932
20. Gray GM, Yardley HJ: Different populations of pig epidermal cells: isolation and lipid composition. J Lipid Research 16:441–447, 1975
21. Gray GM, White RT: Glycosphingolipids and ceramides in human and pig epidermis. J Invest Dermatol 70:336–341, 1978
22. Elias PM, Brown BE, Fritsch P, Geerke JH, Gray MG, White RJ: Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum. J Invest Dermatol 73:339–348, 1978
23. Genstein W: The phospholipids of normal and psoriatic skin. J Invest Dermatol 40:105–109, 1963
24. Camussi G, Aglietta M, Malavasi F, Tetta C, Piacibello W, Wanavio, F, Bussolino F: The release of platelet-activating factor from human endothelial cells in culture. J Immunol 131:2397–2403, 1983
25. Prescott SM, Zimmerman GA, McIntyre TM. Human endothelial cells in culture produce platelet-activating factor when stimulated with thrombin. Proc Natl Acad Sci USA 81:3534–3538, 1984

26. Shier TW, Baldwin JH, Nilsen-Hamilton M, Hamilton RT, Thanassi NM: Regulation of guanylate and adenylate cyclase activities by lysolecithin. Proc Natl Sci USA 73:1586–1590, 1976
27. Cruckhorn P, Willis AL: Cutaneous reactions to intradermal prostaglandins. Br J Pharmacol 41:49–56, 1971
28. Lewis RA, Austen KF: Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. Nature 293:103–106, 1981
29. Ford-Hutchinson AW: Leukotriene involvement in pathologic processes. J Allergy Clin Immunol 74:437–440, 1986
30. Dowd PM, Black AK, Woolard PM, Camp RDR, Greaves MW: Cutaneous responses to 12-hydroxy-5,8,10,14-eicosatetraenoic acid. J Invest Dermatol 84:537–541, 1985
31. Camp RDW, Coutts AA, Greaves MW, Kay AB, Walport MJ: Responses of human skin to intradermal injection of leukotrienes C_4, D_4, and E_4. J Invest Dermatol 78:329, 1982
32. Camp RDW, Jones RR, Brain S, Woolard P, Greaves MW: Production of intraepidermal microabscesses by topical application of leukotriene B_4. J Invest Dermatol 82:202–204, 1984
33. Czarnetzki B: Increased monocyte chemotaxis towards leukotriene B_4 and platelet activating factor in patients with inflammatory dermatoses. Clin Exp Immunol 54:486–492, 1983
34. Morley J, Page CP, Paul W: Inflammatory actions of platelet activating factor (pafeceter) in guinea pig skin. Br J Pharmacol 80:503–509, 1983
35. Lowe NJ, Stoughton RB: Effects of topical prostaglandin E_2 analogue on normal hairless mouse epidermal DNA synthesis. J Invest Dermatol 68:134–137, 1977
36. Cha C, Duhamel L, Ford-Hutchinson A. Leukotriene B_4 and 12-hydroxyeicosatetraenoic acid stimulate epidermal proliferation in vivo in the guinea pig. J Invest Dermatol 85:333–334, 1985
37. Eaglstein H, Ginsberg LD, Mertz PM: Ultraviolet irradiation-induced inflammation. Arch Dermatol 115:1421–1423, 1979
38. Kragballe K, Voorhees JJ: LTB_4 and keratinocyte growth. J Allergy Clin Immunol 74:426–430, 1984
39. Nordlund JJ, Ackles EE: Melanocytotoxins and fatty acids which stimulate proliferation of melanocytes in murine pineal epidermis: a possible role for arachidonic acid metabolism in regulation of melanocyte growth. Banara J, Klaus SN, Paul E, Schartel M (eds.). Biological, Molecular and Clinical Aspects of Pigmentation, Pigment Cell. University of Tokyo Press, Tokyo, 1985, pp. 501–513
40. Nordlund JJ, Collins CE, Rehins LA: Prostaglandin E_2 and D_2 but not MSH stimulate the proliferation of pigment cells in the pineal epidermis of the DBA/2 Mouse. J Invest Dermatol 86:433–437, 1986
41. Ziboh VA, Lord JT, Uematsu S, Blick G: Activation of phospholipase A_2 and increased release of prostaglandin precursor from skin by ultraviolet irradiation. J Invest Dermatol 70:211, 1979
42. Bowser PA, Gray MG: Sphingomyelinase in pig and human epidermis. J Invest Dermatol 70:331–335, 1978
43. Ziboh VA, Lord JT. Phospholipase A activity in the skin. Biochem J 184:283–290, 1979
44. Long VJW, Yardley HJ. Phospholipase A and lysophospholipase activity of the epidermis. Br J Dermatol 70:331–335, 1978

JEFFERSON CENTER FOR INTERNATIONAL DERMATOLOGY RESIDENTS ESSAY CONTEST

The Department of Dermatology at Jefferson Medical College and the Jefferson Center for International Dermatology are pleased to announce the Jefferson Center for International Dermatology Essay Contest in connection with the 4th Intercontinental Congress of Dermatology and Venerology, to be held in Caracas, Venezuela, *February 11–14, 1989.* Residents in the approved dermatology programs anywhere in the world are encouraged to submit original studies relevant to clinical and basic sciences of dermatology. The winner will be invited to present the paper in the meeting in 1989. Please submit the essays, no later than October 1, 1988, to the Chairman of the Judging Committee: Gary P. Lask, M.D., Chief, Division of Cutaneous Surgery, Department of Dermatology, Jefferson Medical College, 1020 Locust Street, Philadelphia, PA 19107