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Alterations in Lectin Binding to the Epidermis Following Treatment with 8-Methoxypsoralen Plus Long-Wave Ultraviolet Radiation

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The alterations in lectin fluorescence stainings to the epidermis were examined in guinea pig skin treated with topical application of a 1% 8-methoxypsoralen (8-MOP) solution plus long-wave ultraviolet (UVA) radiation (1.5–3.5 J/cm²) (PUVA). Serial biopsy specimens taken up to 21 days postirradiation were stained with 8 commercially available lectins labeled with either fluorescein isothiocyanate (FITC) or biotin (followed by avidin D-FITC): *Bandeiraea simplicifolia* agglutinin I (BSA), concanavalin A (Con-A), *Dolichos biflorus* agglutinin (DBA), peanut agglutinin (PNA), *Ricinus communis* agglutinin I (RCA), soybean agglutinin (SBA), *Ulex europeus* agglutinin I (UEA), and wheat germ agglutinin (WGA). In normal guinea pig skin UEA staining was absent. Following PUVA treatment, UEA and DBA stainings became apparent or stronger in intensity after days 7–14 (UEA) and days 4–7 (DBA), respectively, and returned to negative or weak by days 14–21. Stainings with Con-A, SBA, and WGA gave remarkable decreases in intensity after days 2–4 and recovered to the baseline by days 7–14. Intensity of BSA, PNA, and RCA stainings was decreased to a lesser degree than the other lectins. Such changes were not produced by application of 8-MOP, UVA radiation (<10 J/cm²), UVB radiation (900–2700 mJ/cm²), or tape stripping. These results suggest that PUVA treatment perturbs the composition or organization of epidermal cell surface glycoconjugates to induce alterations in lectin stainings.

Psoralen plus long-wave ultraviolet (UVA) radiation (PUVA) has been used successfully in the treatment of several skin diseases including psoriasis, mycosis fungoides, and vitiligo [1]. However, the basic mechanism of PUVA treatment is not fully understood. This is partly because of the complexity of the skin, which consists of a variety of cell populations, structures, and reaction sites. Nuclear DNA certainly is a susceptible target for psoralens [2]. However, psoralen-induced DNA cross-linking and subsequent suppression of DNA synthesis alone do not seem to explain the whole mechanism of action of the photochemotherapy. The inhibitory effect of experimental PUVA on the immune system may be partially attributed to a PUVA-induced alteration in Langerhans cell surface characteristics [3].

We have previously demonstrated that PUVA administered to guinea pig back skin depletes epidermal cell membrane fluorescence with pemphigus antibodies and anti-guinea pig epidermal cell sera (AES), suggesting that PUVA may affect certain cell surface domains, probably glycoproteins [4]. The advent of lectins, which are proteins or glycoproteins originally isolated from plant seeds, has recently allowed more careful investigation of membrane glycoconjugates, including glycoproteins, glycolipids, and extracellular proteoglycans, because of their ability to bind to specific sugar residues [5]. In the present study, histochemical staining with 8 different labeled lectins was undertaken in PUVA-administered guinea pig skin to visualize alterations in cell surface glycoconjugates. The effect of middle-wave ultraviolet (UVB) radiation and tape stripping was also examined.

MATERIALS AND METHODS

Psoralen

A 1% 8-methoxypsoralen (8-MOP) solution was commercially available from Taisho Pharmaceutical Company, Tokyo, Japan. The base of this solution consists of ethanol, acetone, and propylene glycol (7:2:1 in vol).

UV Light Source

We used an instrument with 10 tubes of 20-W fluorescent black light (Toshiba FL 20S BLB). Each tube emits rays of 300–400 nm, peaking at 360 nm (mainly UVA). The total energy output measured by a UV radiometer (Tokyo Kogaku) through a 2 mm-thick glass filter was 4.0 mW/cm² in the range of UVA (320–400 nm) and 0.03 mW/cm² in the range of UVB (300–320 nm) at the distance of 30 cm.

Experimental Design

A total of 32 albino male guinea pigs (Hartley strain, 300–500 g body weight) were used. After plucking and shaving, the left half of the back of an animal was painted with the 8-MOP solution (approximately 10 μL/cm²) (PUVA-treated side). The other side was untreated (control side). One hour later a single shot of UVA radiation (1.5–3.5 J/cm²) was given to the whole skin surface. The individual experiment was repeated 3 times using 8 guinea pigs in a group. The UVA dose range between 2.0–3.0 J/cm² produced approximately 1 minimal phototoxic dose in the guinea pig. Controls also included 6 guinea pigs receiving a single shot of higher UVA energy doses (3.5–10 J/cm²) without application of 8-MOP and 2 guinea pigs receiving a single application of 8-MOP only.

UVB Radiation

Six guinea pigs were irradiated with 5 tubes of fluorescent sunlamp (Toshiba FL 20SE) for 4–12 min (900–2700 mJ/cm²) at a distance of 30 cm. This lamp emits rays between 280–320 nm, peaking at 310–315 nm. The total energy doses administered were equal approximately to 0.5–1.5 minimal erythema doses.

Tape Stripping

One day after plucking and shaving the backs of 3 guinea pigs were stripped using adhesive cellophane tape until discrete glistening of the skin appeared (4–7 times). The treatment has been shown to increase

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Abbreviations:
AES: anti-guinea pig epidermal cell sera
BSA: *Bandeiraea simplicifolia* agglutinin I
Con-A: concanavalin A
DBA: *Dolichos biflorus* agglutinin
FITC: fluorescein isothiocyanate
8-MOP: 8-methoxypsoralen
PBS: phosphate-buffered saline
PNA: peanut agglutinin
PUVA: psoralen plus long-wave UV radiation
RCA: *Ricinus communis* agglutinin I
SBA: soybean agglutinin
UEA: *Ulex europeus* agglutinin I
UVA: long-wave UV radiation
UVB: middle-wave UV radiation
WGA: wheat germ agglutinin

the rate of tritiated thymidine uptake and subsequently results in remarkable epidermal thickening [6].

Biopsy

Biopsy was undertaken before and at various time intervals ranging from 3 h to 21 days after each treatment. Biopsy specimens were immediately frozen in a Dry Ice-acetone mixture and stored at −80°C.

Lectins

Lectins used in this study are summarized in Table I. Fluorescein isothiocyanate (FITC)-labeled *Bandeiraea simplicifolia* agglutinin I (BSA) and soybean agglutinin (SBA) are products of P-L Biochemicals (Milwaukee, Wisconsin). Conjugation and purification of concanavalin A (Con-A)-FITC were done in our laboratories [4]. FITC-labeled *Ulex europeus* agglutinin I (UEA) was purchased from Calbiochem (La Jolla, California).

Biotinylated *Dolichos biflorus* agglutinin (DBA), peanut agglutinin (PNA), *Ricinus communis* agglutinin I (RCA), UEA, and wheat germ agglutinin (WGA) were all purchased from Vector Laboratories (Burlingame, California).

Stain Techniques

Frozen tissues were mounted in a Tissue-Tek II O.C.T. compound (Lab-Tek Products, Division Miles Laboratories, Illinois) and cut into 4-μm sections on a cryostat at −20°C. Sections were air-dried and used without fixation. For FITC-labeled lectins the sections were covered with the reagent for 30 min at 25°C. A biotin/avidin system included the following procedures: The sections were first overlayered with appropriately diluted, biotinylated lectins for 30 min at 25°C, rinsed in phosphate-buffered saline (PBS) pH 7.4, and reacted with FITC-labeled avidin D (diluted to 30–60 μg/mL with PBS, F/p molar ratio 4.9, Vector Laboratories) for 30 min at 25°C. All slide glasses were rinsed again in PBS, mounted in a glycerol:PBS (9:1) mixture, and examined in a Nikon fluorescence microscope with a 200-W high-pressure mercury lamp and epi-illumination ultraviolet optics.

Neuraminidase Pretreatment

Air-dried, unfixed cryostat sections were exposed to *Vibrio cholerae* neuraminidase (Calbiochem) for 30 min at 37°C at a concentration of 0.01–0.5 IU/mL in PBS and then subjected to lectin staining.

Inhibition Studies

Specific, unlabeled competing sugars, including D-galactose for BSA, PNA, and RCA, N-acetyl-D-galactosamine for DBA and SBA, α-L-fucose for UEA, N-acetyl-D-glucosamine for WGA were purchased from Sigma Chemical Company (St Louis, Missouri). For inhibition studies, sections were exposed to a reaction mixture containing a diluted, labeled lectin and an isotonic solution of the corresponding competing sugar adjusted to a final concentration of 200 mM (BSA, Con-A, PNA, and SBA) or 500 mM (others). Staining with avidin D-FITC only did not show any specific fluorescence but occasionally showed a tenuous, nonspecific binding in the junctional area between granular and horny layers, probably due to a high F/p molar ratio (4.9) of the reagent.

Evaluation of Fluorescence Intensity

Six to eight different slide glasses per specimen were prepared for each lectin staining. After all sections were examined, intensity of fluorescence was evaluated by the following method: For each lectin staining, intensity of fluorescence before treatment (positive control) was always determined as + (baseline). Then, ++ indicated a relative increase in intensity as compared to positive controls; +/− a relative decrease in intensity (including all intermediates between + and −); and − completely negative as revealed by inhibition studies (see Figs *2C* and *3D*). However, the baseline for UEA staining was (−), because it was undetectable in normal guinea pig skin (see *Results*). Intensity of fluorescence was not compared between one and another lectin. Several photographs were taken under a settled exposure time using Fujichrome 100 Type D films. Furthermore, using photomicrographs and on the same grading basis, evaluation of intensity of fluorescence was performed by a person without knowledge of the experimental group. Generally, our data were well corroborated by the latter evaluation.

RESULTS

Lectin Binding to Normal Guinea Pig Skin

Table II summarizes the results. In normal guinea pig skin no specific UEA staining was observed in epidermis and dermis using an FITC staining method or a biotin/avidin system (Fig *1A*). Specific fluorescence did not appear after neuraminidase pretreatment. DBA staining was very weak in normal guinea pig skin (Fig *2A*). PNA and SBA preferentially bound to granular and upper spinous layers. Staining with Con-A, RCA, and WGA (Fig *3A*) was seen in the whole epidermis. BSA stained weakly the lower epidermis (Fig *4A*).

A linear fluorescence at the basement membrane zone was detected by at least 3 lectins including Con-A, RCA, and WGA. Dermal components were positive with DBA, SBA, Con-A, BSA, RCA, and WGA; RCA appeared to stain dermal vessels, while the others stained the dermis diffusely. The stratum corneum was occasionally stained with Con-A and WGA. Specificity of the lectin staining was confirmed by the inhibition studies.

TABLE I. *Summary of the lectins used in this study*

Lectin	Conjugated with	Specificity	Working dilution μg/mL
BSA	FITC	α-Gal	500–1000 (F/p = 0.92)
Con-A	FITC	α-Man/α-Glc	100–500 (F/p = 0.65)
DBA	Biotin	α-GalNAc	10–100
PNA	Biotin	β-Gal	5–100
RCA	Biotin	β-Gal	10–100
SBA	FITC	α-GalNAc	250–500 (F/p = 0.44)
UEA	FITC/biotin	α-L-Fuc	20–250 (F/p = 1.2)/10–100
WGA	Biotin	GlcNAc	5–100

TABLE II. *Lectin binding to normal guinea pig skin*

Lectin	Stratum corneum	Epidermis			Basement membrane	Dermal components
		Upper/mid	Whole	Lower		
UEA	—		−		−	−
DBA	—	+			±	+
PNA	—	+			−	−
SBA	—	+			−	+
BSA	—			+	−	+
Con-A	±		+		+	+
RCA	−		+		+	+
WGA	±		+		+	+

In this table, − indicates negative; ± tenuous fluorescence and partially negative; and + positive (including weak to remarkable fluorescence).

Alterations in Lectin Staining Following a Single PUVA Treatment

Table III summarizes the kinetics of epidermal lectin staining after administration of 3.0–3.5 J/cm^2. Energy doses of 1.5–2.0 J/cm^2 produced substantially similar alterations in fluorescence intensity but seemingly to a lesser degree.

After days 4–7 of PUVA, fluorescence for UEA became detectable mainly in midepidermis and increased gradually in intensity, peaking on day 7 (1.5–2.0 J/cm^2) or day 14 (3.0–3.5 J/cm^2) (Fig 1*B*,*C*, negative control). Fluorescence for UEA became negative by day 21 but in a few specimens (4/24 animals) detectable fluoresence persisted to the last day of the experimental period. While DBA stained untreated skin very weakly, it depicted an amplified intensity of fluorescence in the upper epidermis in PUVA-treated skin. Fluorescence intensity became most obvious after days 4–7 (Fig 2*B*,*C*, negative control) and returned to the baseline by day 14. Fluorescence for UEA was always seen in the midepidermis, while DBA staining was always confined to the upper epidermis: there was no recognizable movement of fluorescence-positive areas with time.

Fluorescence for SBA, Con-A, and WGA decreased in intensity after PUVA treatment most notably between days 2–4 (Fig 3*B*, WGA) and recovered by day 7 (1.5–2.0 J/cm^2) or 14 (3.0–3.5 J/cm^2) (Fig 3*C*,*D*, negative control). Changes in the lectin binding were not apparent in the early phase, i.e., by 3 h postirradiation. Larger energy amounts seemed to produce more pronounced and longer suppression in fluorescence intensity. No changes were noticed in fluorescence patterns on days 7–21 as compared to those in positive controls (see Fig 3*C*). Alterations in fluorescence pattern and intensity of the basement membrane and dermal components were generally much less conspicuous than those in the epidermal living layers.

Intensity of fluorescence for BSA, PNA, and RCA was also decreased 2–7 days after PUVA radiation (Fig 4*B*, BSA) but changes were seemingly less remarkable than those of SBA, Con-A, and WGA. No notable changes were seen in the basement membrane and dermal fluorescence.

There were no detectable alterations in lectin fluorescence following either topical application of 8-MOP or distribution of as high energy doses of UVA as allowed in this study (1.5–10 J/cm^2).

TABLE III. *Alterations in intensity of lectin staining fluorescence in epidermis following PUVA treatment*

Lectin	Before treatment	Days after PUVA[a] treatment				
		2	4	7	14	21
UEA	−	−	−	++	++	−
DBA	+	+	++	++	+	+
SBA	+	+/−	+/−	+/−	+	+
Con-A	+	+/−	+/−	+/−	+	+
WGA	+	+/−	+/−	+/−	+	+
BSA	+	+/−	+/−	+/−	+	+
PNA	+	+/−	+/−	+	+	+
RCA	+	+	+/−	+	+	+

[a] 3.0–3.5 J/cm^2.

In this table, + indicates positive controls; ++ relative increases in intensity; +/− relative decreases in intensity; and − negative (for details, see the *Materials and Methods*).

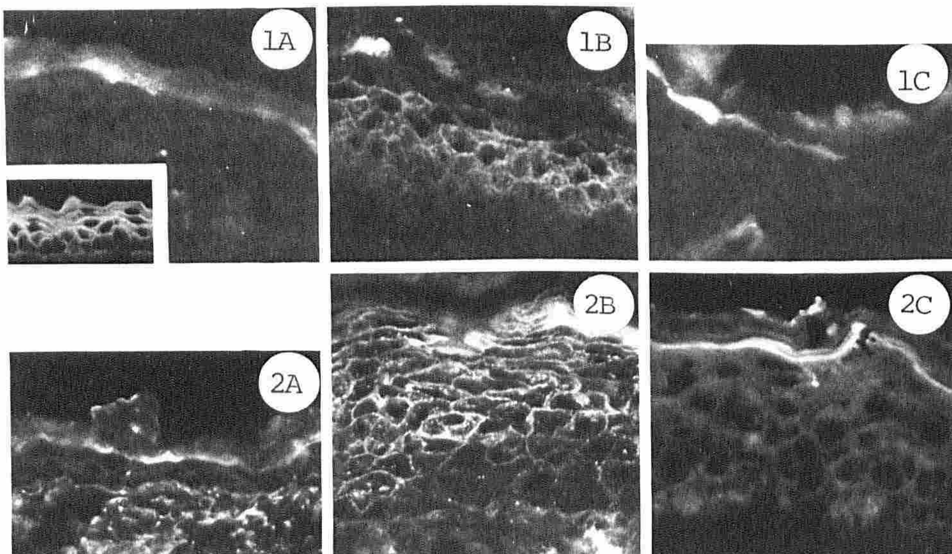
Lectin Staining after UVB Radiation and Tape Stripping

Specimens from UVB-irradiated skin were stained with only 3 lectins: UEA, Con-A, and WGA. There were no obvious alterations in intensity and pattern of fluorescence following either treatment. UEA staining was not recognized throughout the observed period.

DISCUSSION

In normal guinea pig skin, lectin exhibited specificity for cell surface moieties of different epidermal populations, as has been shown in human [7], mouse [8], and newborn rat [9] skin. However, absence of UEA staining in guinea pig epidermis appeared to be a species-specific profile of the surface carbohydrate composition and agreed with the finding that UEA does not bind to cultured guinea pig epidermal cells [10]. It is likely that L-fucose residues, UEA-specific sugar, are either absent or present in amounts undetectable by fluorescence microscopy. Pretreatment of cryostat sections with neuraminidase failed to restore UEA staining, suggesting that this sugar moiety was not masked by sialic acid which often terminates oligosaccharide chains of both glycoproteins and glycolipids [11].

Intensity of fluorescence in SBA, Con-A, and WGA stainings decreased after 2–4 days and recovered gradually to the baseline of intensity by days 7–14. BSA, PNA, and RCA gave decreased intensity in fluorescence staining to a lesser degree. Generally, kinetics of these lectin stainings appeared to be similar to those of other markers for glycoproteins of epidermal cell membranes, such as pemphigus antibodies and AES [4]. Fluorescence staining with these antibodies decreases notably in intensity 2–4



FIGS 1 and 2. Biotinylated UEA and DBA stainings. *1A*, Absence of UEA staining in normal guinea pig skin (20 μg/mL). Compare to a positive UEA staining in upper epidermis in normal mouse ear skin (*inset*). *1B*, Appearance of UEA staining after 14 days of PUVA radiation (3.0 J/cm^2). Note that fluorescence is localized mainly on cell membranes and in the cytoplasm of the acanthotic midepidermis. *1C*, Abolishment of UEA staining in *B* by addition of α-L-fucose (500 mM) to the reaction mixture (negative control). *2A*, A weak DBA staining in normal guinea pig skin (40 μg/mL) (positive control). *2B*, Enhanced epidermal DBA staining after 7 days of PUVA radiation (2.0 J/cm^2). A granular fluorescence is also seen in the cytoplasm. *2C*, Abolishment of DBA staining in *B* by addition of N-acetyl-D-galactosamine (500 mM) to the reaction mixture (negative control). Unabsorbed fluorescence seen in the junctional area between granular and horny layers appears to be nonspecific. (× 500)

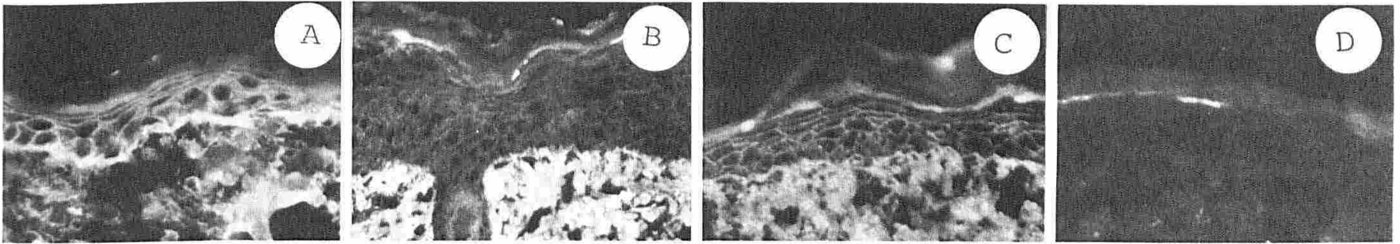


FIG 3. Biotinylated WGA staining. *A*, A positive WGA staining in normal guinea pig skin (25 μg/mL) (positive control). *B*, WGA staining with remarkably decreased intensity of epidermal fluorescence after 2 days of PUVA treatment (3.0 J/cm²). *C*, Recovery of epidermal WGA staining after 14 days of PUVA treatment. *D*, Abolishment of positive WGA staining in *C* by addition of N-acetyl-D-glucosamine (500 mM) to the reaction mixture (negative control). Unabsorbed fluorescence seen in the junctional area between granular and horny layers appears to be nonspecific. (× 500)

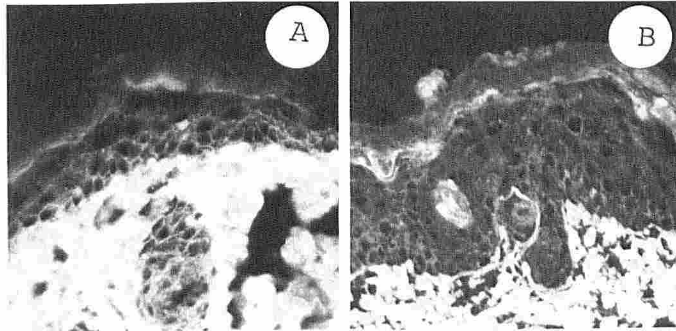


FIG 4. Fluorescinated BSA staining. *A*, A positive but weak staining seen mainly in the lower epidermis of normal guinea pig skin (positive control). *B*, BSA staining with decreased intensity after 4 days of PUVA treatment (3.0 J/cm²). (× 500)

days after PUVA radiation and returns to the baseline by days 6–8. Delayed recovery of lectin stainings as compared to that of the antibody stainings may be partially due to different turnover rates of membrane glycoproteins.

It was speculated that decreases in intensity of lectin stainings resulted from degradation of membrane glycoproteins induced by PUVA through photodynamic or oxygen-mediated type II reactions [12,13] or by release of cell surface proteases or lysosomal enzymes. Wennersten [14] has used scanning electron microscopy to demonstrate that a single administration of PUVA radiation to in vitro cultured cells caused conspicuous membrane damage by 2 h postirradiation, although an intensive PUVA regimen was used ($>10^{-4}$ M for 8-MOP, 5 J/cm² of UVA radiation). In our study, decreases in intensity of lectin stainings became apparent 1–2 days after PUVA treatment. This latent period does not seem to be explained by acute membrane damage by PUVA alone. PUVA is known to suppress epidermal DNA synthesis within the first few hours postirradiation with subsequent recovery by 24 h [15]. Therefore, it is possible also that decreases in intensity of lectin stainings may result from a transient perturbation of membrane macromolecule synthesis following interference with the central metabolic system by PUVA.

In contrast, fluorescence with UEA and DBA appeared or was enhanced after PUVA and these changes coincided with recovery from transient suppression of fluorescence intensity of other lectin stainings. Thus, it is suggested that a certain modulation of biosynthesis of epidermal membrane glycoproteins occurs at the recovery stage from acute membrane damage caused by PUVA, resulting in precipitation on cell membranes of detectable amounts of particular sugars specific for UEA and DBA. Such perturbation or redistribution of lectin binding seems to take place also in retinoid-treated mouse ear epidermis. Nemanic, Fritsch and Elias [16] have shown that while UEA normally stains only the granular layer, it depicts the entire viable epidermis after 17–20 days of oral retinoid uptake. The distribution of PNA, WGA, and succinyl WGA is also affected.

UVB radiation and tape stripping did not apparently alter the intensity and pattern of lectin fluorescence stainings throughout the course of epidermal injury and subsequent hyperproliferation. Therefore, PUVA-induced kinetics of lectin stainings does not reflect simply a phenomenon that is nonspecifically seen in the hyperproliferating epidermis. Rather, the variations of lectin fluorescence stainings seem to represent a unique photochemical event, although the clinical significance of our findings remains to be elucidated.

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