Effects of PUVA Therapy on Skin Surface Lipids: Skin Surface Lipid Peroxidation in Psoriasis Vulgaris and its Biological Significance

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PUVA therapy (Psoralen + UVA irradiation) is an effective mode of photochemotherapy for psoriasis vulgaris. The biological significance of PUVA therapy for psoriasis vulgaris has mainly been considered to be based on DNA, especially the formation of DNA crosslinks between complementary DNA strands. On the other hand, we have already reported that skin surface lipids were oxidized by UVA irradiation in vitro and this reaction was enhanced in the presence of 8-methoxy-psoralen by a singlet oxygen mechanism. Keeping this in mind, we conducted an experiment to determine whether lipid peroxide can be formed in skin surface lipids following systemic PUVA therapy in psoriasis patients and the following results were obtained: 1) a marked increase of lipid peroxide values in skin surface lipids occurred following PUVA therapy; and 2) the amount of squalene in skin surface lipids was decreased by this treatment. These results indicate that skin surface lipids can be oxidized by PUVA therapy in vivo and this lipid peroxidation on the skin surface may be related to the effects of PUVA therapy on psoriasis vulgaris.

(Key Words: PUVA Therapy, Psoralen, UVA, Skin surface lipids, Squalene, Lipid peroxide)

INTRODUCTION

The skin surface is covered with a mixture of lipids (skin surface lipids) derived from epidermal cells and sebaceous glands. Skin surface lipids have an important role in protecting and shielding the epidermis from environmental damage and exert an inhibitory action on cutaneous insensible perspiration (7). On the other hand, it has been proved that irradiation of skin surface lipids by sunlight, including ultraviolet light, can lead to the formation of lipid peroxides through a photooxidation mechanism in some of the unsaturated lipids (12).

PUVA therapy is a kind of photochemotherapy using long wavelength ultraviolet light (UVA) irradiation following systemic or topical use of psoralen(P), especially 8-methoxypsoralen (8-MOP). 8-MOP is stimulated to produce the 8-MOP radical by UVA irradiation and this, in turn, produces singlet oxygen \( (1\text{O}_2) \) by excitation of molecular oxygen to a higher energy state(3). We have already reported the in vitro peroxidation of squalene, which is a major component of polyunsaturated lipids in skin surface lipids, by UVA irradiation in the presence of 8-MOP (4, 13). With this in mind, we conducted an experiment to determine whether lipid peroxide can be formed in vivo on the skin surface following systemic PUVA therapy in psoriasis patients, and whether lipid peroxidation on the skin surface is related to the effects of PUVA therapy. We analyzed lipid composition and the amount of lipid peroxides in skin surface lipids before and after PUVA therapy.

MATERIALS AND METHODS

Patients

We selected four males (ranging from 28 to 45 years old) who had psoriasis involving more than 50 percent of the body surface, and two normal males (21 and 27 years old) as controls.

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Light source and PUVA therapy

The M-DMR-AL type Dermaray (Toshiba-Eisai) was used as a UVA source. This device was equipped with 72 lamps (FL 20s BLB: Toshiba, ranging from 300 to 430 nm in wavelength with a sharp peak at 365 nm and a broad peak at 352 nm). The light fluence rate was measured with a Toshiba UV-radiometer (Model UVA-356). The total single dosage of UVA irradiation was set at 24 J/cm². Psoriasis patients were irradiated with UVA one hour after the ingestion of 8-MOP (40 mg), while the normal healthy males received only UVA treatment.

Collection of skin surface lipids

Skin surface lipids were collected using the cup method. An open-ended cup with an area of 12.56 cm² was applied to the skin surface. Five milliliters of acetone was poured into the cup and one minute later the lipid-containing acetone was collected. This sample collection was repeated twice on each area. In the psoriasis patients, two symmetrical areas of equal involvement on the trunk were chosen and the samples were collected from one side before PUVA therapy and from the other after PUVA therapy.

Measurement of the concentration of lipid peroxide

The quantity of lipid peroxide was measured using the thiobarbituric acid (TBA) method(12). An aliquot of the solution containing skin surface lipids was dried under N₂ gas flow immediately after collection and was suspended in 0.2 ml of 8 % sodium dodecyl sulfate (SDS). To the suspension, 1.5 ml of 20 % acetic acid (pH 4.0), 1.5 ml of 0.67 % TBA (Wako Pure Chemical) and 0.8 ml of distilled water were added, and the mixture was then incubated in a hot bath at 95 °C for 60 minutes. After cooling, 4 ml of butanol was added and the mixture was shaken. The butanol layer was separated by centrifugation, and its spectrophotometric absorption at 532 nm was measured with a Hitachi 239 digital spectrophotometer. When the amount of TBA reactive substance was too small to be measured by spectrophotometry, it was measured by spectrofluorometry with excitation at 515 nm and fluorescence at 550 nm (spectrofluorometer FP-550A, Nihon Bunkoh). Tetraethoxypropane (Tokyo Kasei) was used as a standard substance to prepare the calibration curve. In the tables and figures, the concentration of TBA-reactive substances was tentatively expressed as the entire concentration corresponding to tetraethoxypropane.

Measurement of the lipid fraction

An aliquot of the collected sample was transferred to an aluminum dish. Total lipids in the sample were measured quantitatively by an ultramicrobalance (Shimadzu LM 20). The lipid fraction was analyzed by thin layer chromatography (TLC) (Silicagel G, Merck). Following the development of the samples, the TLC plate was soaked in a 10 % sulfuric acid solution with 0.04 % potassium perchromic acid, and thereafter it was heated at 120 °C for 30 minutes. The concentration of each lipid was calculated by densitometry (Shimadzu dual wave chromatoscanner CS-900). The following systems were used in development of the chromatoplates: I)n-hexane, 18.5 cm, II) benzene, 18.5 cm and III) ethylether:n-hexane: acetic acid (92:18:1), 7.5 cm. These systems enabled us to analyze squalene (SQ), cholesterol ester (CE), wax monoester, wax diester, triglyceride (TG), free fatty acid (FFA) and free cholesterol (Ch)(1). The unit used was µg/cm².

RESULTS

Effects of PUVA therapy on skin surface lipid peroxidation

The lipid peroxide values in skin surface lipids obtained from the involved area of psoriasis patients just before and immediately after PUVA therapy are given in Table 1. Three samples from three distinct sites were collected from each of the four psoriasis patients.

Despite initial differences in lipid peroxide values of the skin involved in different patients, there was a notable increase in lipid peroxide values following PUVA therapy. This increase was statistically significant in each case since the p-value was less than 0.05. The mean lipid peroxide value increased by a factor of 2.8.

In contrast, the mean lipid peroxide values in two normal controls to whom no 8-MOP was given were 0.35 n mol/10 cm² and 0.55 n mol/10 cm² before and immediately after UVA irradiation, respectively. The mean increase rate
was 1.9-fold.

The lipid peroxide values in the skin surface lipids in one psoriasis patient were followed after PUVA therapy (Fig. 1). A high lipid peroxide value was noticed even before PUVA therapy. This lipid peroxide value had increased on hour after PUVA therapy to five times that of the pretreatment level and then slowly decreased to the pretreatment level. When a normal healthy man was irradiated by UVA at the same dosage as a psoriasis patient but without 8-MOP, the increase in the lipid peroxide value was slight and the level returned to normal in two hours (Fig 2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>MDA formed Before (n mol/cm²)</th>
<th>MDA formed Immediately After (n mol/cm²)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>0.17</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>0.19</td>
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<tr>
<td>4</td>
<td>0.27</td>
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<td>5</td>
<td>0.28</td>
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<tr>
<td>6</td>
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<tr>
<td>7</td>
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<tr>
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</table>

![Fig. 1](Image)

**Fig. 1** Lipid peroxide values of the skin surface lipids following PUVA therapy in one psoriasis patient (40 mg of 8-MOP administered systemically).
Effects of PUVA therapy on skin surface lipid composition

Fig. 3 shows the effects of PUVA therapy on skin surface lipid composition in three psoriasis patients, since we failed to collect sufficient skin surface lipids in one psoriasis patient. Some of the skin lesions of psoriasis patients showed higher levels of free cholesterol and cholesterol ester than normal skin before PUVA therapy. These high values may be due to the thick horny layer which is rich in free cholesterol and cholesterol ester. However, the levels of all components of skin surface lipids were decreased by PUVA therapy. This decline might be attributable to degradation of surface lipids through oxidation by PUVA therapy. The lipid composition of surface lipids in normal controls remained unaltered by UVA irradiation (Fig. 4).

Fig. 3 Amount of lipid fraction in the skin surface lipids of three psoriasis patients before and immediately after PUVA therapy (8-MOP: 40mg, UVA: 24J/cm²). Two lipid samples were collected from one patient.
DISCUSSION

Lipid peroxidation was performed on the skin surface lipids by PUVA therapy and the levels of squalene, free cholesterol and cholesterol ester were reduced. Squalene which has six double bonds and is formed in the sebaceous glands makes up about 10% of skin surface lipids (8). Skin surface lipids also contain triglycerides, wax, wax ester, free cholesterol, cholesterol ester, phospholipids and free fatty acids (5). About 50% of the fatty acid composition of triglycerides, wax ester and cholesterol ester are unsaturated. However, the percentage of dienes which have a pair of double bond is only 3% compared with 47% of monoens in the fatty acids of skin surface lipids, and the oxidation potential of dienes is also low due to their stability compared to polyunsaturated lipids with multiple double bonds (11). The ratio of oxidation of triolein, trilinolen and trilinolein is 1:120:330 (11). These facts suggest that the lipid peroxide produced by PUVA therapy on skin surface lipids is mainly derived from squalene, which has six double bonds in its chemical structure, while little is derived from unsaturated fatty acids. The decrease in squalene level following PUVA therapy may represent a process of degradation via lipid peroxide formation by PUVA therapy.

We have already reported that squalene produced lipid peroxide by UVA irradiation in vitro (12), and this reaction was enhanced in the presence of 8-MOP by the singlet oxygen mechanism (4, 13). Considering these facts, the mechanism of lipid peroxide formation on the skin surface is postulated as follows. The ingested 8-MOP is stimulated in epidermal tissue by absorption of photons and the stimulated 8-MOP reacts directly with some molecules in the skin, i.e., nucleic acids, proteins, lipids or other biomolecules. In other cases, the triplet stimulated state of 8-MOP generates singlet oxygen by energy transfer. Singlet oxygen can oxidize unsaturated lipids. These facts suggest that stimulated 8-MOP was formed in the skin following UVA irradiation and it led to lipid peroxidation in vivo, with or without the singlet oxygen mechanism. The amount of lipid peroxide in the skin surface lipids was increased and the amount of free cholesterol and cholesterol ester was decreased by PUVA therapy. Psoriatic scales contain approximately 53.6% cholesterol (10) in their lipid fraction. These results suggest that cholesterol ester and free cholesterol are also oxidized (9) on the skin surface by PUVA therapy.

The increased lipid peroxide values in skin surface lipids of psoriasis patients returned to pretreatment levels by 4 hours after PUVA ther-
apy. These results also suggest that the epidermis has inhibitory effects on the accumulation of lipid peroxide or the degradation mechanism of lipid peroxide on the skin surface as we have already proposed (14).

The biological significance of PUVA therapy against psoriasis vulgaris has been mainly discussed on the basis of effects on DNA, especially the formation of DNA crosslinks between complementary DNA strands (2). However, our results suggested that lipids are one of the target molecules of PUVA therapy of psoriasis vulgaris in addition to DNA.

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REFERENCES