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ORIGINAL ARTICLE

Evaluation of apoptosis regulatory proteins in response to PUVA therapy for psoriasis

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SUMMARY

Background

The histopathologic changes characteristic of psoriasis might be related to suppressed apoptosis. One of the actions of psoralen ultraviolet A (PUVA) in psoriasis could be exerted through induction of apoptosis of keratinocytes and lymphocytes; however, its exact molecular mechanism is still confusing.

Aim

In this study, we evaluated the expression of pro-apoptotic (P53, Fas and Bax) and anti-apoptotic (Bcl-2) proteins correlating it with apoptotic index (AI) and epidermal thickness in psoriatic skin before and after PUVA therapy.

Methods

Lesional and non-lesional skin biopsy specimens were obtained from 10 patients with generalized plaque psoriasis before and after 8 weeks of PUVA therapy. Histometric measurements of epidermal thickness as well as P53, Fas, Bax and Bcl-2 expressions were evaluated using immunoperoxidase technique and apoptotic cells were detected by terminal deoxynucleotide transferase (TdT) mediated deoxyuridine triphosphate nick end labeling (TUNEL) method.

Results

After PUVA therapy, the epidermal thickness of psoriatic skin was significantly decreased ($P < 0.001$) and keratinocytes of psoriatic skin showed significant increased expression of P53 ($P < 0.001$), Fas ($P < 0.001$) and Bcl-2 ($P < 0.001$) with no significant change in Bax expression ($P > 0.05$). Apart from significant decrease of Bcl-2 expression ($P = 0.01$), no significant difference in all previous markers were encountered in lymphocytes (P53, Fas and Bax; $P > 0.05$) after PUVA therapy. The AI was significantly increased ($P = 0.008$) after PUVA therapy especially in lymphocytes ($P = 0.002$).

Conclusion

The present study suggests that one of the actions of PUVA therapy in psoriasis might be exerted through induction of apoptosis especially of lymphocytes by suppression of Bcl-2 expression and of keratinocytes through P53 and Fas pathways leading to healing of psoriasis.

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The etiopathogenesis of psoriasis is not fully understood. However, T lymphocytes play a crucial role in the induction and maintenance of psoriatic lesions (1). Meanwhile, it has been postulated that increased epidermal thickness in psoriasis may be related to an abnormality in the apoptotic pathway (2).

Apoptosis, or programmed cell death, is a physiologic, genetically encoded program that results in cell death maintaining proliferative homeostasis (3). In controlling cellular proliferation, the wild type of P53 (tumor-suppressor gene) can cause cell cycle arrest in response to DNA damage allowing time for DNA repair or cell destruction by an irreversible apoptotic pathway before replication renders the damage permanent (4). Fas (CD95), a membrane protein, is a member of tumor necrosis factor receptor (TNFR) family. Cross-linking of Fas by its ligand, FasL, induces apoptosis of cells expressing Fas on the membrane by triggering a cascade of caspases (5).

Several proteins of the Bcl-2 gene family are involved in the regulation of programmed cell death either by preventing (Bcl-2, Bcl-xl, Bcl-w, Bcl-B, Bfl-1 and Mcl-1) or by promoting apoptosis (Bax, Bik, Bak, Bad, Bcl-Xs, Bim, Noxa and Puma). Bcl-2 gene, encoding an inner mitochondrial protein, protects cells from apoptosis by binding to the Bax protein. Bax (Bcl-2-associated X protein), a protein with approximately 21% sequence homology with Bcl-2, has been reported to be present predominantly in the cytosol redistributing from its soluble to mitochondrial membrane-bound form in cells undergoing apoptosis (6).

Psoralen ultraviolet A (PUVA), using oral psoralen and ultraviolet A radiation, is known as a highly effective treatment for moderate to severe psoriasis because of its antiproliferative, anti-inflammatory and immunosuppressive effects (7). Moreover, it has been reported by many investigators that PUVA induces apoptosis of both keratinocytes and lymphocytes leading to healing of psoriasis (2, 8).

The present study aimed to evaluate the expression of pro-apoptotic (P53, Fas and Bax) and anti-apoptotic (Bcl-2) proteins correlating it with apoptotic index (AI) and epidermal thickness in psoriatic skin before and after PUVA therapy.

PATIENTS AND METHODS

Patients

The study was conducted on 10 patients with generalized plaque psoriasis and was approved by the Committee for Postgraduate Studies and Research of Al-Minya University. The patients did not receive topical, systemic antipsoriatic treatment or phototherapy for at least 3 months before starting the study. Informed consent was taken from each patient for photographing, treating with PUVA therapy and taking biopsies.

PUVA therapy

Considering the guidelines of the British Photodermatology Group (9), patients received PUVA sessions twice/week until complete remission, or for a maximum period of 16 weeks. Two hours before UVA exposure, each patient ingested ultramicronized 8-methoxypsoralen (8-MOP) at a dose of 0.6 mg/kg body weight/session (Ultra-Meladinine capsules; 10 mg capsules, Memphis Pharmaceutical Co., Cairo, Egypt). Then, the patient was irradiated in a UVA compact cabin (GP-42, Cosmedico Medizintechnik, Villingen-Schwenningen, Germany). The initial irradiation dose was calculated according to the skin type of the patient. All patients were skin type IV-V. Accordingly, the initial dose was 2–2.5 J/cm^2 and the dose was increased by 40%/week until erythema occurred, then increased by 20%/week with a maximum of 15 J/cm^2. The severity of psoriasis was evaluated using the PASI (Psoriasis Area and Severity Index) score according to Fridriksson and Pettersson (10) before treatment and at every session up to 16 weeks.

Biopsy

For evaluating apoptotic proteins, biopsies were taken, nearly from the same sites, before treatment and after 8 weeks from starting treatment, because initial clinical improvement was usually noticed around the eighth week of treatment and before the complete remission of the disease. Each time, two biopsies were obtained using 4-mm punch from a sun-protected area; one from a psoriatic plaque (lesional biopsy) and the other from normally appearing perilesional skin (non-lesional biopsy) at least 15 cm away from the psoriatic lesions. All biopsies were fixed in 10% buffered formalin, embedded in paraffin and sectioned into 5-μm-thick sections. These sections were subjected to; histopathological examination using hematoxylin and eosin (H&E) stain and immunohistochemical examination. Light microscope [Accu-Scope # 3025 five-headed (A3025-5), Olympus, Tokyo, Japan] with a built-in camera (digital camera E-330 SLR, Olympus) was used to examine and photograph the sections.

Histometric evaluation of epidermal thickness

A computer assisted program (analySIS®Five Olympus Soft Imaging Solutions GmbH, Johann-Krane-Weg 39, D-48149, Munster, Germany) was employed to measure epidermal thickness in H&E stained sections of lesional and non-lesional biopsy specimens before and after

8 weeks of PUVA therapy. The mean epidermal thickness was determined by measuring the distance between the outermost surface of the epidermis excluding stratum corneum and the dermo-epidermal junction at 5 points through the entire length of three examined sections.

Immunohistochemical examination

Lesional and non-lesional biopsy specimens, before and after 8 weeks of PUVA therapy, were stained by four rabbit monoclonal mouse antihuman antibodies [P53 protein (code no.: RMPD 016, ready-to-use 1ry antibody, DBS, Pleasanton, CA, USA), Fas (code no.: M3554, DAKO Corporation, Carpinteria, CA, USA; at a dilution of 1: 30), Bax (code no.: E3381, ready-to-use 1ry antibody, Spring Bioscience, Fremont, CA, USA) and Bcl-2 oncoprotein (code no.: PDM 016, ready-to-use 1ry antibody, DBS)].

Each of the previous primary antibodies was applied to cover each section, and then slides were placed in a humidity chamber and incubated for 2 h. The ready-to-use detection system (code no.: K0673, DAKO LSAB2 system, DAB, DAKO Corporation) was used to demonstrate antibodies expression according to the manufacturer instructions.

The level of all apoptosis markers expression in keratinocytes was evaluated, by two blinded histopathologists, in accordance with the scoring system devised by Liang *et al.* (4). This system results in a score ranging from 0 to 3 for both the degree of positivity (percentage of positively stained epidermal cells: 0; < 1%, 1; 1–10%, 2; 10–50%, 3; > 50%) and the intensity of staining [from faint-brown (score 1) to deep-brown (score 3)]. The sum of the two scores was taken as the level of expression.

Expression of apoptotic markers in dermal lymphocytes was evaluated according to Yildiz *et al.* (11) in a score ranging from 0 to 4 as follows: 0, none; 1, < 25%; 2, 26–50%; 3, 51–75%; 4, > 75% staining of the lymphocytes.

For detection of apoptotic cells, sections from lesional biopsy specimens of all patients before and after 8 weeks of PUVA therapy were stained by the terminal deoxynucleotide transferase (TdT)-mediated deoxyuridine triphosphate nick end labeling (TUNEL) method using *in situ* apoptosis detection kit (code: KGA7031, ready to use, KeyGen Biotech 3F, Nanjing, China). AI was evaluated according to Kikuchi and Nishikawa (12) as follows:according to Kikuchi and Nishikawa (12) as follows:

$$\text{AI}\,(\%) = (\text{TUNEL positive cells}/\text{total cells counted}) \times 100$$

All tissue sections were stained under similar conditions to ensure equal staining quality. Negative control sections were done by omitting the primary antibody in the staining technique. Meanwhile, positive controls for P53, Bcl-2, Bax and Fas were obtained by staining serous ovarian carcinoma, follicular lymphoma, desmoplastic small cell carcinoma and human prostate tissue respectively. The positive control for TUNEL method was obtained by staining human thymus tissue.

Statistical analysis

Data were statistically analyzed using SPSS for Windows, Version 16.0.1 (Chicago, IL, USA). Statistical analysis included descriptive analysis as mean ± standard deviation (SD), paired and independent-samples *t*-test and correlation coefficient (r) for the results. Significance was expressed in terms of *P*-value, which was considered significant when it was ≤0.05.

RESULTS

The study included 10 patients with generalized plaque psoriasis (7 males and 3 females). Their age ranged from 28 to 55 years (mean, 41.6 ± 8.6 years). Eight patients were skin type IV and two patients were skin type V. The duration of psoriasis ranged from 3 months to 23 years (mean, 8.3 ± 8.0 years). The cumulative UVA dose ranged from 171 to 187 J/cm2 (mean, 176.1 ± 4.5). Before starting treatment, PASI score ranged from 6.0 to 26.2 (mean, 16.0 ± 6.1). After 8 weeks from starting treatment, the PASI score was significantly reduced to range from 4.1 to 13.1 (mean, 9.3 ± 3.0, $P = 0.001$), while after 16 weeks from starting treatment, it was further reduced ranging from 0.1 to 8.4 (mean, 2.4 ± 2.6, $P < 0.001$).

Histopathological examination

Before treatment, lesional skin biopsy specimens revealed the characteristic features of psoriasis. After 8 weeks of treatment, these features were reduced with more improvement in the epidermal than the dermal changes.

Histometric evaluation of epidermal thickness

The epidermal thickness of lesional biopsy specimens of psoriatic patients ranged from 147.74 to 293.17 μm (mean, 220.96 ± 52.55 μm), that was statistically significantly higher if compared to non-lesional skin ($P < 0.001$). After 8 weeks of treatment, the epidermal thickness was significantly decreased (mean, 72.14 ± 19.72 μm; $P < 0.000$) (Fig. 1). On the other hand, the epidermal thickness of non-lesional biopsies ranged from 56.30 to 79.20 μm

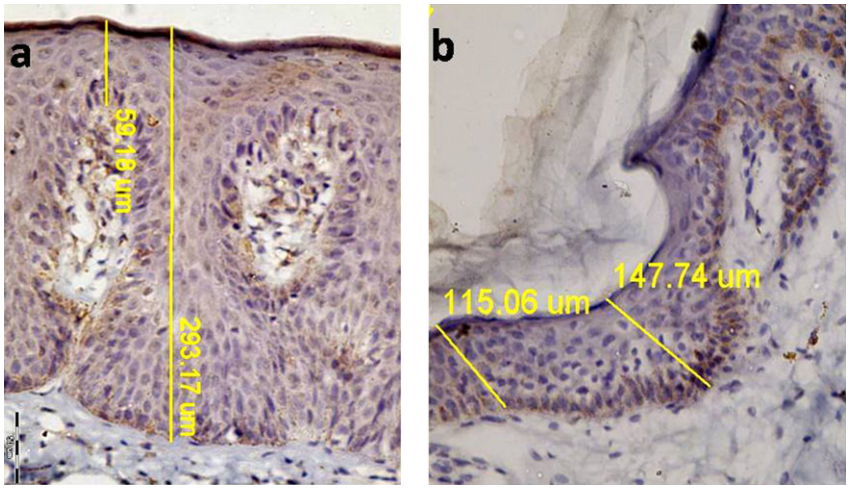

Fig. 1. Histometry. (a) Psoriatic lesion before PUVA treatment showing increased epidermal thickness. (b) After 8 weeks of PUVA therapy, the epidermal thickness was significantly decreased (H & E; ×400).

(mean, 66.79 ± 6.55 μm) with no statistically significant difference when compared to post-treated biopsies (mean, 66.77 ± 6.54 μm; $P > 0.05$).

Immunohistochemical examination

Lesional biopsy specimens

After PUVA therapy, the keratinocytes of psoriatic skin showed significant increase in expression of P53 ($P < 0.001$), Fas ($P < 0.001$) and Bcl-2 ($P < 0.001$) with no significant change in Bax expression ($P > 0.05$) (Table 1). Apart from significant decrease of Bcl-2 expression in lymphocytes ($P = 0.01$), no significant difference in all previous markers in lymphocytes (P53, Fas and Bax; $P > 0.05$) were encountered after PUVA therapy. There were no apoptotic keratinocytes or lymphocytes detected in psoriatic plaques before treatment. After 8 weeks of PUVA therapy, the AI was significantly increased in keratinocytes ($P = 0.008$) and lymphocytes ($P < 0.001$). However, the AI of lymphocytes was higher than that of keratinocytes ($P = 0.002$) (Table 1 & Figs 2–4).

It is worth mentioning that P53 positive cells demonstrated nuclear staining with dispersed pattern in all positive biopsies. Fas positive expression appeared as membranous staining of all Fas-positive specimens. As regards Bax and Bcl-2 expression, it appeared as a cytoplasmic staining pattern in all positive biopsy specimens.

Non-lesional biopsy specimens

Comparing to normal non-lesional skin, psoriatic keratinocytes showed statistically significant increase in P53 (mean; 0.05 ± 0.09, $P < 0.001$), significant decrease in Bcl-2 (mean; 0.9 ± 0.5, $P = 0.03$) with no significant difference in the expression of both Fas and Bax (Fas, 0.03 ± 0.07 & Bax, 0.02 ± 0.06, $P > 0.05$). After 8 weeks of PUVA therapy, non-lesional keratinocytes showed significant increase in both P53 (mean, 0.8 ± 0.8, $P = 0.01$) and Bcl-2 expression (mean, 1.1 ± 0.6, $P = 0.02$); however, both Fas (mean, 0.04 ± 0.1, $P > 0.05$) and Bax expression (mean, 0.15 ± 0.3, $P > 0.05$) were not significantly increased. Lymphocytes were negative for all previous markers before and 8 weeks after PUVA therapy.

Apart from significant positive correlation between cumulative UVA dose and the level of Fas and Bax in lymphocytes after PUVA therapy ($P < 0.05$), there was no significant correlation between UVA dose, P53, Bcl-2 and increased apoptotic cells ($P > 0.05$). As regards PASI score, there was no statistically significant correlation with either pro-apoptotic or anti-apoptotic markers after 8 weeks of PUVA therapy ($P > 0.05$). Moreover, there were no statistically significant correlations ($P > 0.05$) between any of pro-apoptotic (P53, Fas and Bax) and anti-apoptotic (Bcl-2) proteins, or with either the AI or the epidermal thickness after 8 weeks of therapy.

DISCUSSION

Psoriasis is recognized as a primarily inflammatory disorder induced and sustained by skin infiltrating lymphocytes with a secondary striking proliferation of keratinocytes and epidermal hyperplasia (13), which produced mainly from aberrant epidermal expression of apoptosis related molecules leading to suppression of the apoptotic process (14).

Previous studies reported over-expression of P53 in the keratinocytes of lesional skin of psoriatic patients (11, 15) than non-lesional skin (16–18). This was explained as a physiological reaction to hyperproliferation and not to

Table 1. Expression of pro-apoptotic and anti-apoptotic markers in psoriatic keratinocytes and lymphocytes before and after 8 weeks of PUVA therapy

	P53		Fas		Bax		Bcl-2		Apoptotic index	
$n = 10$	Before	After	Before	After	Before	After	Before	After	Before	After
Keratinocytes										
Range	0.0–3.0	2.5–4.5	0–0.3	1.5–3.2	0–0.4	0 –0.3	0.0–1.5	1.2–2.4	0	0–2.4
Mean	1.8 ± 1.0	3.2 ± 0.5	0.07 ± 0.12	2.33 ± 0.6	0.11 ± 0.14	0.1 ± 0.1	0.4 ± 0.6	1.7 ± 0.4	0	1.2 ± 1.0
Negative biopsies	2	0	7	0	5	3	7	0	10	4
P	$P < 0.001$		$P < 0.001$		$P > 0.05$		$P < 0.001$		$P = 0.002$	
Lymphocytes										
Range	0.0–2.0	0.0–2.0	0–0.3	0–0.4	0–0.2	0–0.3	0.0–2.0	0.0–1.0	0	0–13.2
Mean	0.3 ± 0.6	0.3 ± 0.6	0.06 ± 0.1	0.08 ± 0.13	0.03 ± 0.07	0.06 ± 0.1	0.8 ± 0.7	0.1 ± 0.3	0	6.5 ± 4.12
Negative biopsies	8	8	7	6	8	7	4	9	10	2
P	$P > 0.05$		$P > 0.05$		$P > 0.05$		$P = 0.01$		$P < 0.001$	

DNA repair (18, 19). The results of the present study agree with previous reports. Meanwhile, the weak expression of P53 in non-lesional keratinocytes may be attributed to taking all skin biopsies from sun-protected areas, which usually show low P53 expression if compared to sun-exposed skin (20).

As regards Fas and Bax expression in keratinocytes, the non-significant difference between lesional and non-lesional biopsy specimens agrees with El-Domyati *et al.* (21). Meanwhile, these results contradict with other studies, which showed over-expression of Fas (14, 22, 23) and Bax (14, 24, 25) in lesional keratinocytes when compared to non-lesional keratinocytes. In lymphocytes, Fas and Bax were expressed to a lesser extent in three and two lesional biopsy specimens, respectively. On the other hand, Tomkova *et al.* (26) observed numerous Bax-positive, infiltrating lymphocytes in all psoriatic specimens. It is worthy to note that there were no comments in previous reports about Fas expression in psoriatic lymphocytes.

Opposing the action of previous apoptotic markers (P53, Fas and Bax), actively proliferating cells typically express Bcl-2 that protects them against apoptotic stimuli, while terminally differentiated cells lose Bcl-2 expression. In the skin, basal keratinocytes usually express Bcl-2 and suprabasilar keratinocytes do not (15, 27). In psoriatic keratinocytes and lymphocytes, controversial labeling results with anti-Bcl-2 antibodies have been observed in which Bcl-2 was under-expressed in keratinocytes (15, 24, 28, 29) and over-expressed in lymphocytes of psoriatic skin (11, 28, 30). Our results are in accordance with those findings and because of the unexpected limited expression of Bcl-2 in keratinocytes, it is thought not to be directly associated with keratinocyte proliferation or apoptotic resistance in psoriasis (28, 31). Meanwhile, an over-expression of Bcl-2 in lymphocytes of psoriatic skin may lead to prolonged survival of lymphocytes resulting in the relapsing and chronic characteristics of psoriasis (11, 28, 30).

In the present study, the absence of apoptotic keratinocytes and lymphocytes in pre-treated lesional biopsy specimens agrees with Kastelan *et al.* (32), who suggested that psoriatic keratinocytes have a phenotype that resists apoptosis. In previous literature, the presence of apoptotic keratinocytes in psoriatic skin varied from the complete absence (14, 28) to the presence of TUNEL-positive cells either in decreased (33) or increased number (15).

In response to PUVA therapy, human epidermal keratinocytes and lymphocytes showed an inhibition of proliferation both *in vitro* (34) and *in vivo* in lesional psoriatic tissue, however, the precise pathways and molecules involved remain poorly unidentified (35). Accordingly, post-treated psoriatic plaques showed reduction in

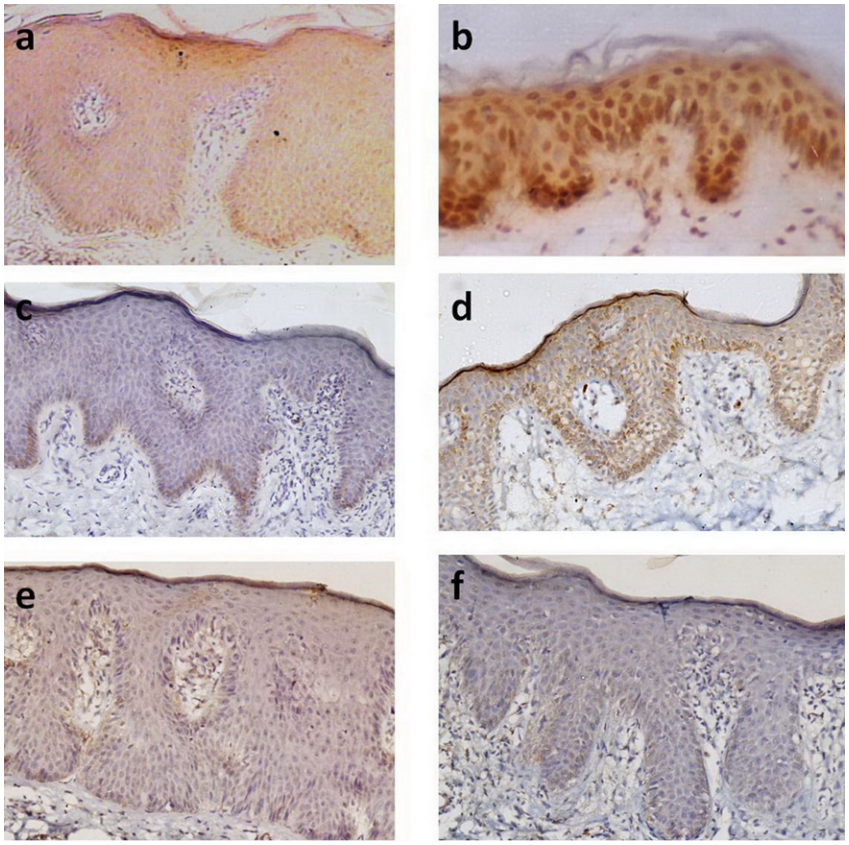

Fig. 2. Immunohistochemical staining of pro-apoptotic markers; P53, Fas and Bax. Psoriatic lesion before PUVA treatment (a, c, e) showing minimal P53 expression (a), negative Fas and Bax expression in the epidermis (c, e). After 8 weeks of PUVA treatment (b, d, f), nuclear staining of P53 expression (b) and membranous staining of Fas expression (d) are significantly increased in both basal and squamous cell layers with no significant change in Bax expression (f) (Original magnification; ×200).

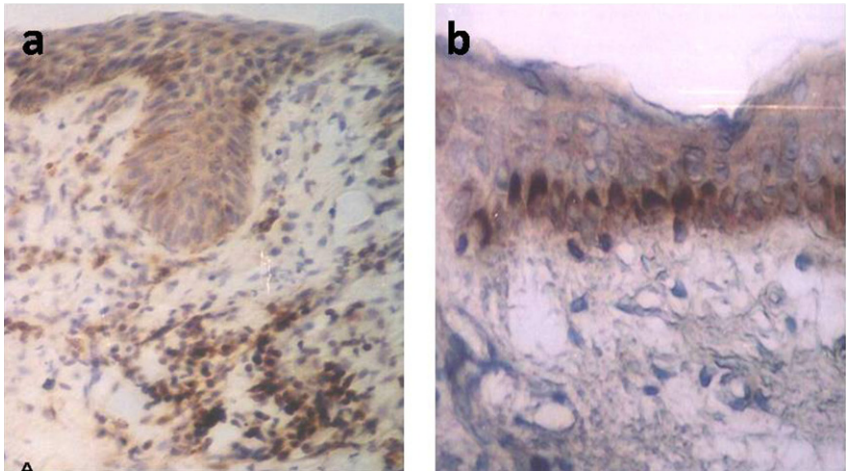

Fig. 3. Immunohistochemical staining of anti-apoptotic protein; Bcl-2. (a) Psoriatic lesion before treatment showing Bcl-2 expression in lymphocytes while the epidermis is negative. (b) After 8 weeks of PUVA therapy, there is no Bcl-2 expression in lymphocytes while the basal keratinocytes show Bcl-2 expression (Original magnification; ×400).

epidermal thickness, parakeratosis and lymphocytic infiltrate (36). Our results agree with the previous work; however, the improvement in the epidermis was more obvious than the dermis. In addition, the use of histometry technique confirmed that the epidermal thickness of lesional biopsy specimens was significantly decreased after 8 weeks of PUVA therapy. To the best of our knowledge, there were no previous reports in the literature concerning the effect of PUVA therapy on the epidermal thickness, measured by the histometry technique, in patients with psoriasis.

After 8 weeks of PUVA therapy, a significant increase of P53 expression in keratinocytes of both lesional and non-lesional biopsy specimens is in accordance with Hannuksela-Svahn *et al.* (16), who concluded that increased P53 expression in non-lesional keratinocytes is

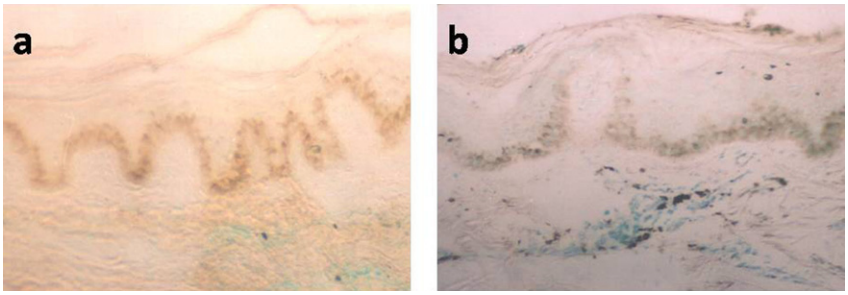

Fig. 4. Immunohistochemical staining with TUNEL. (a) Psoriatic lesion before treatment showing no apoptotic cells. (b) After 8 weeks of PUVA, there are apoptotic lymphocytes (TUNEL; a; ×100, b; ×200).

likely to be induced by DNA damage caused by PUVA while in psoriatic lesions; it could be a result of the combined effect of increased epidermal proliferation and DNA damage. Previous *in vitro* studies showed that most DNA-damaging agents induce apoptosis via a p53-mediated pathway, thereby reducing the risk of accumulation of genetically aberrant cells (37).

Significant increase of Fas expression in post-treated lesional keratinocytes agrees with Leverkus *et al.* (38), who suggested that Fas system contributes to keratinocyte apoptosis in ultraviolet rays irradiated human skin *in vitro*. On the other hand, Bax was not significantly increased after 8 weeks of PUVA therapy when compared to pre-treatment level in both keratinocytes and lymphocytes. To the best of our knowledge, there were no previous reports in the literature concerning the level of Fas or Bax in both psoriatic keratinocytes and lymphocytes after PUVA therapy.

Comparing with pre-treated biopsies, Bcl-2 expression in post-treated biopsies was significantly increased in the keratinocytes of both lesional and non-lesional skin. Meanwhile, it was significantly decreased in lymphocytes of lesional skin. There was only one report discussing the effect of PUVA therapy on Bcl-2 expression in psoriatic lesions, in which there was down regulation of Bcl-2 level in psoriatic lymphocytes without any changes in psoriatic keratinocytes after PUVA therapy (39). As Bcl-2 is not incriminated in the epidermal proliferation or apoptotic resistance in psoriasis (31), the increased epidermal Bcl-2 expression after PUVA therapy may be attributed to restoration of normal basal cell activity because Bcl-2 expression may control the increased apoptosis induced by PUVA. On the other hand, the suppressed Bcl-2 expression in lymphocytes after PUVA therapy leads to apoptosis and thus promotes healing of psoriatic plaques.

Generally, ultraviolet rays induce apoptosis to both keratinocytes and lymphocytes at the same degrees (35). However, it has been found that UVB is a stronger inducer of epidermal apoptosis than UVA (40). On the other hand, PUVA was found to be more potent in inducing apoptosis in lymphocytes than in keratinocytes (41). Moreover, some investigators reported that PUVA selectively induces apoptosis of lymphocytes (8, 42) at doses that do not harm keratinocytes (3). This can be explained by the fact that UVB delivers most of the energy to the epidermis, whereas UVA penetrates deeper into the skin (42).

After 8 weeks of PUVA therapy, we detected apoptotic keratinocytes and lymphocytes in the lesional biopsy specimens. This is in accordance with Laporte *et al.* (2) and Weatherhead *et al.* (15), who reported that healing of psoriatic plaques is associated with apoptosis. However, PUVA appears to induce apoptosis mainly in lymphocytes rather than keratinocytes, as the AI of lymphocytes was significantly higher than that of keratinocytes.

The present study showed significant increase of both P53 and Fas expression with concomitant increase in the AI in psoriatic keratinocytes after PUVA therapy, confirming that PUVA induces apoptosis of psoriatic keratinocytes through both P53 and Fas dependent pathways. This is in accordance with Santamaria *et al.* (43), who stated that PUVA treatment induces apoptosis in mouse epidermal cells both *in vitro* and *in vivo* through activation of both p53 and Fas, in which P53 induces Fas expression and then activates caspase-3, ultimately resulting in apoptosis or through activation of either of them independently (44). The previous suggestion is supported by an interesting possibility, in which psoralens exert double actions both in the nucleus and on mitochondria (37). Accordingly, PUVA may induce apoptosis via multiple pathways.

Dermatologists have been concerned about the increase in incidence of squamous cell carcinoma observed in psoriatic patients treated with PUVA (45) as mutation of the P53 gene was considered as an important factor in the pathogenesis of ultraviolet light-induced skin cancer (46, 47). It was found that the pattern of P53 staining in the skin reflects the type of present protein, whether dispersed in wild-type or compact in mutated type (48). In the present study, the pattern of P53 staining was found to be

of the dispersed type in all positive biopsies, whether before or after PUVA therapy, suggesting that P53 is of the wild-type. This is in accordance with the results of El-Domyati *et al.* (17) and Yazici *et al.* (18). Accordingly, mutation analysis of P53 is mandatory especially when mutated P53 is expected as after sun exposure and PUVA therapy (49, 50).

In conclusion, the present study suggests that one of the actions of PUVA therapy in psoriasis might be exerted through induction of apoptosis especially of lymphocytes by suppression of Bcl-2 expression and of keratinocytes through P53 and Fas pathways. However, PUVA appears to induce apoptosis of lymphocytes more than keratinocytes, thus promoting healing of psoriatic lesions.

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