

308 nm MONOCHROMATIC EXCIMER LIGHT IN PSORIASIS: CLINICAL EVALUATION AND STUDY OF CYTOKINE LEVELS IN THE SKIN

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Ultraviolet light (UVB, 290-320 nm), and in particular light at 308-311 nm, represents an effective therapeutic modality for psoriasis and other immunomediated skin disorders. In fact, its therapeutic mechanism has been attributed to immunosuppressive properties in the skin. Alterations that affect cytokine network related to the abnormal level and activities of T lymphocytes in the altered skin, are considered fundamental today in the pathogenesis of the disease. The aim of our study is to evaluate clinical aspects and cytokine levels in the skin before and after treatment with a 308-nm Monochromatic Excimer Light (Excilite DEKA - Florence - Italy) in psoriatic patients. At 3-month follow-up clearance of treated lesions is still evident.

INTRODUCTION

Psoriasis vulgaris is a common immunomediated disease of the skin. Chronic plaques with well-defined borders and silvery scaling constitute its most characteristic lesions, but many other clinical aspects can be observed.

Ultraviolet light (UV) and UVB in particular (290-320 nm) are effective therapeutic modalities for the treatment of psoriasis (1) and of other immunomediated skin disorders. Their therapeutic mechanism has been attributed to immunosuppressive properties. Recently, in addition to narrow-band UVB (311 nm), a new UVB source generated by a 308-nm excimer laser has been introduced for the treatment of psoriasis (2-4). The aim of our study is both clinical evaluation and evaluation of cytokines levels in the skin before and after treatment with a 308-nm Monochromatic Excimer Light (MEL).

PATIENTS, METHODS AND QUANTITATIVE PCR STUDY

Patients. Eighty-one subjects were recruited (46 M and 35 F, age range 19-67) of which 70 af-

ected by plaque-type psoriasis vulgaris and 11 with palmoplantar psoriasis. Patients were required to discontinue both topical treatments and photochemotherapy for 4 weeks and all systemic treatments for at least 6 months before enrollment.

Methods. The 308 nm XeCl MEL (EXCILITE-DEKA - Florence - Italy) produces a power density of 48 mW/cm² at the distance of 15 cm from skin and a maximum irradiating area of 512 cm² (Fig. 1). Before the beginning of treatment, 308-nm UVB minimal erythema dose (MED) was determined on uninvolved dorsum skin with an increasing time of exposure. Fourteen days before MEL treatment was started a 4 mm punch biopsy sample was obtained from lesional skin under local anesthesia after informed consent in a selected group of 5 patients and was snap-frozen in liquid nitrogen and stored at -80°C until it was prepared for polymerase chain reaction (PCR).

After protecting non affected skin, each psoriatic area was irradiated (Fig. 2). The first treatment session started from the MED time (range 8-15 seconds) and increased by 3-10 seconds during the following sessions with a maximum of 90 seconds. Each patient underwent a variable number of

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Fig. 1. 308 nm excimer light (Excilite).

treatment sessions (from 1 to 15). The treatment sessions were performed 2 or 3 times a week. Treatment was concluded when the clearance of lesions had been occurred. When lesions appeared clinically cleared, another biopsy was obtained from the same area with the same modalities. Nothing was applied to the plaques of psoriasis before treatment with the MEL. Clinical evaluation was done every

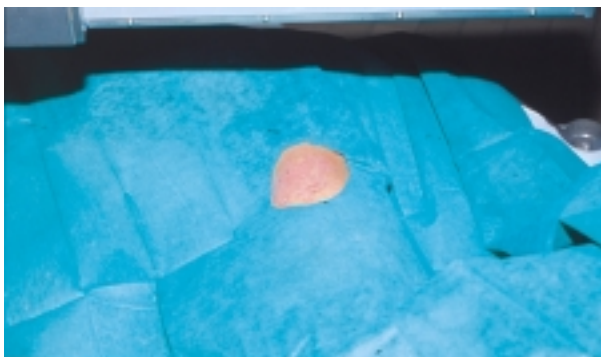


Fig. 2. Psoriasis of the leg during Excilite treatment.

2 weeks during the period of therapy and on the first, second, third month after the last irradiation. No additional treatment except a petrolatum cream was used during the study period.

Quantitative PCR study. Total RNA extraction with RNAwiz (Ambion, Austin, TX) was performed on ten specimens of skin (pre- and post-treatment). Total RNA was reverse transcribed with cDNA Cycle Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Six experiments of DNA amplification were performed using PCR Light Cycler (Idaho Technology, Idaho Falls, ID). The Light Cycler system consists of a micro-volume fluorimeter integrated with a thermal cycler that combines rapid PCR cycles in glass capillaries heated with hot air with real time fluorescence monitoring (5-7). Sequence of specific hybridization probes can be designed for the detection and analysis of PCR products (Tab. I). MWG-Biotech (Germany) synthesised and purified primers and probes. PCR was performed with 0.5 mM primers in a standard PCR reaction supplemented with BSA; 0.2 mM fluorescein-labelled probe, 0.2 mM BODIPY-labelled probe BODIPY-labelled probe in 5' and 0.5 U Klen Taq DNA polymerase were mixed in final volume of 10 μ l. Samples were capped and placed in the Light Cycler. Forty cycles of denaturation (94°C, 0 s, ramp rate 20°C/s), annealing (59°C, 15 s, ramp rate 20°C/s) and extension (74°C, 0 s, ramp rate 0.8°C/s) were performed for cytokines study [γ -interferon (INF- γ), interleukin-8 neutrophil activating factor (IL-8/NAP), interleukin-6 (IL-6), α -transforming growth factor (TGF- α) and α -tumour necrosis factor (TNF- α)].

RESULTS

As evidenced by flattening of plaques, decreased scaling and erythema all patients showed clinical improvement (variable from 50% to 100%) after 1-15 sessions (Fig. 3a, 3b, 4a, 4b, 5a, 5b, 6a, 6b). No relapses have been detected except for six patients (partial relapse). Unwanted side effects such as pain or blistering were not observed. Minimal erythema and itching (mostly affecting leg lesions) and a transitory and modest hyperpigmentation were noted in all patients. After remission, the levels of cutaneous cytokines evaluated in the study showed equivalent levels to those of non affected skin as compared to levels before treatment (Fig. 7).

Tab. I. Specific sequences of oligonucleotides and probes used for Light Cycler quantitative PCR study.

Probes and oligonucleotides	Specific sequences
β -Actin forward	5'-TGACGGGGTCACCCACACTGTGCCATCT
β -Actin reverse	5'-TGGAAGCAGCCGTGCCATCTCTTGCTCGA
β -Actin fluorescein probe	5'-CTGGCCGGGACCTGACATGACTACCTCATGA(fluo)-3'
β -Actin Bodipy probe	Bodipy -5'-GACCTCACCGAGCGGGCTACAGCTTCAC(P)-3'
IL-6 forward	5'-AAGATTCCAAAGATGTAGCCGCCACACA
IL-6 reverse	5'-TCTGCCAGTGCCTCTTTGCTGCTTCACAC
IL-6 fluorescein probe	5'-GACAGCCACTCACCTCTTCAGAACGAATTG(fluo)-3'
IL-6 Bodipy probe	Bodipy -5'-CAAACAAATTCGGTACATCCTCGACGGCA(P)-3'
IL-8 forward probe	5'-CCACCCCAAATTTATCAAAGAACTGAGAGT
IL-8 reverse probe	5'-GAATTCTCAGCCCTCTTCAAAAACTTCTCC
IL-8 fluorescein probe	5'-GCTTTCTGATGGAAGAGAGCTCTGTCTGGA(fluo)-3'
IL-8 Bodipy probe	Bodipy -5'-CCCAAGGAAAAGTGGGTGCAGAGGGTGTG(P)-3'
TGF- α forward	5'-TTCCACACTCAGTTCTGCTTCCATGCAAC
TGF- α reverse	5'-CAGCACACATGTGATGATAAGGACAGCCAG
TGF- α fluorescein probe	5'-TGCAGGTTTTTGGTGCACGAGGACAAGCCA(fluo)-3'
TGF- α Bodipy probe	Bodipy -5'-CATGTGCTGCCATCTGGGTACGTTGGTG(P)-3'
TNF- α forward	5'-GACCTCTCTAATCAGCCCTCTGGCCAG
TNF- α reverse	5'-CTGATGGCACCACCAGCTGGTTATCTCTCA
TNF- α fluorescein probe	5'-CCTCAAGCTGAGGGGACAGCTCCAGTGGCTG(fluo)-3'
TNF- α Bodipy probe	Bodipy -5'-ACCGCCGGCCAATGCCCTCCTGGCCAATG(P)-3'
IFN- γ forward	5'-GCTCTGCATCGTTTGGGTT
IFN- γ reverse	5'-CTCCACACTTTTTGGATGC
IFN- γ fluorescein probe	5'-GTAGCGGATAATGGAAGCTTTTCTTAGGC(fluo)3'
IFN- γ Bodipy probe	5'-Bodipy-5'-TTTGAAGAATTGAAAGAGGAGGTGACAG(P)-3'

**Fig. 3a.** Right and left elbow before treatment.**Fig. 3b.** Right elbow 3 months after the end of treatment (twelve sessions, maximum time of exposure: 90"); left elbow after two sessions (12" and 15").**Fig. 4a.** Dorsum of hands before treatment.**Fig. 4b.** Two months after the end of therapy (fourteen treatment sessions, maximum time of exposure: 70").



Fig. 5a. *Small psoriatic lesion of the back.*



Fig. 5b. *One month after a single treatment (15").*



Fig. 6a. *Psoriasis of the knees.*

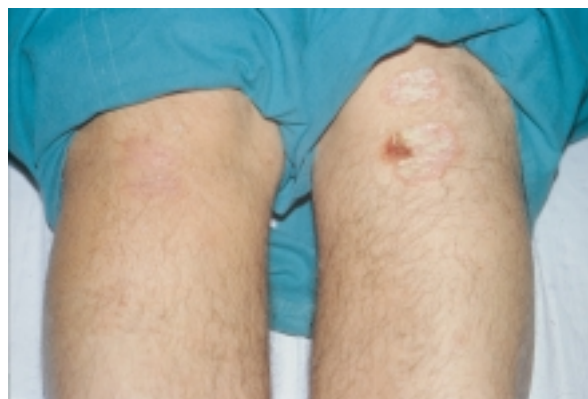


Fig. 6b. *Right knee at three months after last Excilite treatment (15 treatment sessions, maximum time of exposure: 90"). Left knee not treated.*

DISCUSSION

The immunosuppressive properties of phototherapy, in particular of 311 nm UVB (8) and more recently of 308 nm excimer light, seem to be responsible for the temporary clearing of psoriatic lesions.

Psoriatic inflammation can be considered the result of a particular Th1 lymphocytic immunoreactivity that develops on a polygenic basis with successive keratinocyte involvement (9). Alterations that affect cytokine network are considered fundamental in the pathogenesis of the disease (10).

Cytokines are synthesised and released by lymphocytes, monocytes/macrophages, polymorphonuclears, keratinocytes, fibroblasts, endothelial cells, etc. They act as soluble signals of cellular cooperation and among them INF- γ IL-8/NAP, IL-6, TGF- α and TNF- α (that were all hyperexpressed in psoriatic lesions), play a fundamental role in the pathogenesis of psoriasis (Tab. II).

Thus, the prominent current hypothesis is

that inflammation and keratinocytes hyperproliferation in psoriatic lesions result from the abnormal release of these mediators probably induced by the local presentation of still unidentified antigens (11) (streptococcal, mycobacterial or retroviral proteins). It has also been suggested that keratinocyte-mononuclear cell interactions are of central importance in the pathogenesis of psoriasis. Lymphocytes attracted by locally produced chemotactic factors may release γ -interferon (INF- γ) which in turn may induce HLA-DR and intercellular adhesion molecule 1 (ICAM-1) expressions on keratinocytes. ICAM-1 expression may then mediate the binding of mononuclear cells to keratinocytes, so that resulting cellular activation could induce the release of inflammatory and growth-promoting agents (12). This hypothesis is supported both by the discovery of ICAM-1 expression on keratinocytes in psoriatic lesions (13) and by the observation in our samples of high levels of INF- γ . Besides INF- γ , also the other cytokines selected in our study seem to be involved in the pathogenesis of the disease

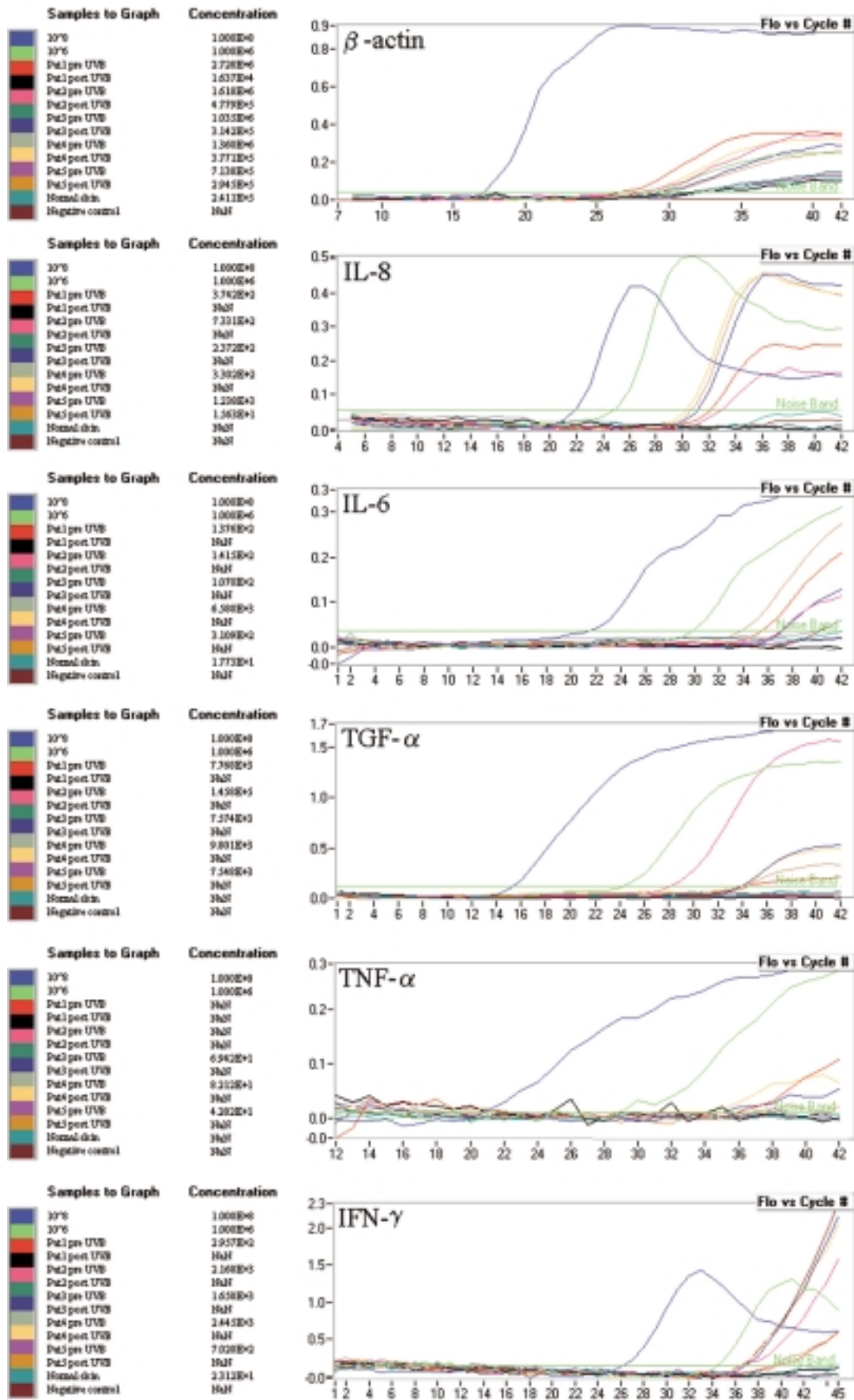


Fig. 7. Pre and post treatment cytokine levels.

Tab. II. *Principal cytokines involved in psoriasis pathogenesis.*

INF- γ	Produced by Th1 lymphocytes. It causes ICAM-1 and HLA-DR expressions on keratinocytes (which are induced to produce TGF- α). Intradermal injection in healed skin of psoriatic patients produces psoriatic lesions (12).
IL-8 NAP-1	Produced by monocytes/macrophages and keratinocytes. It provides chemotactic action towards polymorphonuclears and stimulates keratinocytes proliferation (11,15).
IL-6	Produced by monocytes/macrophages, keratinocytes, fibroblasts and endotheliocytes. Increased in plasmatic level. It stimulates keratinocytes proliferation and T lymphocytes activation and proliferation (16).
TGF- α	Produced by keratinocytes either spontaneously or after stimulus from IL-6, INF- γ e TNF- α . It stimulates keratinocytes proliferation and causes angiogenesis (17).
TNF- α	Produced by macrophages and keratinocytes. It stimulates adhesion molecules expression on keratinocytes and endotheliocytes. It increases epidermal growth factor receptor expression on keratinocytes and TGF-a and IL-8 production by keratinocytes (16).

(Tab. II). The 308 nm MEL (Excilite) plays an essential role in drastically decreasing cytokine expression in psoriatic skin which is accompanied by clinical remission. MEL seems to represent a new safe and effective photo-treatment of psoriasis.

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