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The effect of phototherapy on systemic inflammatory process in patients with plaque psoriasis

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ABSTRACT

Psoriasis is a common, chronic immune-mediated inflammatory disease. The inflammatory process in psoriasis has systemic effects and may influence the development of psoriatic comorbidities. The systemic action of phototherapy in patients with psoriasis has been so far poorly elucidated. We aimed to investigate the expression of genes encoding selected psoriasis-related cytokines in peripheral blood mononuclear cells (PBMCs) isolated from patients with psoriasis before and after treatment with phototherapy. 17 patients with mild to moderate plaque psoriasis were treated with narrow band-UVB (NB-UVB), 8 patients with moderate to severe plaque psoriasis with bath-psoralen-ultraviolet A therapy (PUVA). PBMCs were isolated by Ficoll gradient density centrifugation. Expression of genes encoding TNF-α, IL-17A, IL-6, IL-1 β, INF-γ, and IL-10 in PBMCs of patients with psoriasis before and after phototherapy was analyzed with quantitative RT-PCR. Treatment with NB-UVB therapy led to a significant decrease in IL-17A, TNF-α, and IL-6 mRNA levels in PBMCs ($p = 0.003$; $p = 0.042$; $p = 0.019$, respectively). Following treatment with bath-PUVA therapy, we observed a significant decrease in TNF-α and IL-6 mRNA levels in PBMCs ($p = 0.031$, $p = 0.035$, respectively).
Treatment with phototherapy in patients with psoriasis may affect systemic inflammation by downregulation of the expression of genes encoding proinflammatory cytokines in PBMCs, implicated in the development of psoriasis and psoriatic comorbidities.

1. Introduction

Plaque psoriasis is a common, chronic immune-mediated inflammatory disease that manifests on skin as well-demarcated, erythematosquamous plaques. The severity of disease is typically graded as mild or moderate-to-severe based on skin involvement. The pathogenesis of psoriasis is not completely elucidated. Activation of Th17-mediated immune responses in the skin plays an important role. Increasing number of evidence indicates that the inflammatory process, especially in moderate to severe psoriasis is not only limited to the skin but has systemic character and consequences. Psoriasis is associated with numerous comorbidities including occlusive cardiovascular disease, diabetes, dyslipidemia, obesity and metabolic syndrome that may increase disease burden. Increased levels of proinflammatory cytokines including TNF-α, IL-17A, IL-6, IL-1 β, IL-8, which are implicated in the pathogenesis of both psoriasis and psoriatic comorbidities have been found in lesional skin and circulation of patients with psoriasis. According to the recently proposed concept of the psoriatic march, systemic inflammation results in insulin resistance and endothelial dysfunction, finally increasing the cardiovascular risk in psoriasis [1–5]. Therefore, it is postulated that treatment of psoriasis should aim not only at a clearance of skin lesions but also reduce systemic inflammation. Early therapeutic intervention in patients with psoriasis may be beneficial and prevent the development of above mentioned systemic consequences [3,6].

Phototherapy is an established and effective first-line treatment option for moderate-to-severe psoriasis or mild psoriasis not sufficiently responding to topical therapy Narrowband ultraviolet B therapy (NB-UVB) employs a wave length of 311 nm. PUVA combines the topical or systemic application of 8-methoxypsoralen with ultraviolet A irradiation, inducing DNA interstrand cross-links which inhibit cell proliferation and protein formation. Both modes of phototherapy are effective in moderate-to-severe psoriasis [7,8]. The direct effect of NB-UVB is mostly restricted to the epidermis and upper part of papillary dermis. It reduces the numbers of epidermal Langerhans cells (LC) and dermal dendritic cells (DCs), suppresses Th1 and Th17 signaling pathways and normalizes expression of numerous genes associated with epidermal proliferation and differentiation [9,10]. Since UVA penetrates deeper into the skin than NB-UVB, PUVA acts on epidermis and dermis. PUVA downregulates several proinflammatory cytokines in the skin (e.g. IL-2, IL-6, IL-8, IL-10, TNF-a, INF-g, IL-12p40 and IL-23p19) and Th1 and Th17 signaling pathways [8,11–14]. Furthermore, a few studies

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indicated that phototherapy may exert certain impact on systemic immune response in patients with psoriasis [15–19]. However, the influence of NB-UVB or bath-PUVA on systemic inflammatory process in patients with psoriasis as well as its possible mechanisms of systemic action has not been completely elucidated yet. The aim of the current study was to investigate the impact of NB-UVB and bath-PUVA on expression profile of genes encoding selected pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells (PBMCs) of patients with psoriasis. We further discuss the mechanism of possible action of phototherapy on systemic inflammation in psoriasis.

2. Material and Methods

2.1. Study Cohort

The study cohort consisted of 25 patients with plaque psoriasis undergoing phototherapy at the phototherapy unit of Department of Dermatology and Allergology, Ludwig-Maximilian University, Munich, Germany. For all procedure informed patient's written consent was obtained. The study was conducted according to Declaration of Helsinki Principles and approved by the local ethical committee. Demographic and clinical data, such as age, gender, presence of cardiovascular risk factors (arterial hypertension, dyslipidemia, diabetes, obesity and smoking habit) were collected. Exclusion criteria were other skin diseases, chronic infections, malignancies, chronic liver, renal or cardiovascular diseases, and age under 18 years. Patients had not received any topical or systemic anti-psoriatic therapy at least 1 month prior to the initiation of phototherapy. Patients with mild and moderate psoriasis were selected for NB-UVB phototherapy, whereas individuals with moderate to severe psoriasis were selected for bath-PUVA phototherapy. The severity of skin involvement was assessed with using Psoriasis Area and Severity Index (PASI) by qualified dermatologists before and after treatment. The group treated with NB-UVB consisted of 17 patients (5 females/12 males) aged from 24 to 72 years (mean ± SD = 46.8 ± 17.2 years). The majority of patients suffered from moderate psoriasis, 14 individuals had PASI ≥10 (82.3%). Among recruited patients: 6 patients had controlled arterial hypertension (35.3%), 7 patients had controlled dyslipidemia (41.2%), 1 patient had controlled diabetes (5.8%), 4 patients were obese (23.5%) and 7 subjects reported smoking habit (41.2%). The mean PASI score before NB-UVB therapy was 12.6 ± 2.9 (ranging from 7.3 to 14.8). NB-UVB irradiations (311 ± 2 nm) were administered using a Waldman cabinet (Waldmann Medizintechnik, Germany). NB-UVB was performed according to the guidelines [7]: three to four times per week, for approximately 5–6 weeks (total 16–20 irradiations); starting from the initial dose 0.1–0.3 J/cm^2, that was gradually increased by 0.1 J/cm^2 depending on skin response until a maximum dose (1.8–2.2 J/cm^2) was reached. The cohort treated with bath-PUVA consisted of 8 patients (2 female/6 males), aged from 22 to 61 years (mean ± SD = 43.7 ± 15.2 years). Majority of patients suffered from moderate to severe psoriasis; 5 patients had PASI ≥10 and 2 patients had PASI ≥20. The mean PASI score before bath-PUVA therapy was 16.5 ± 6.6 (ranging from 8.3 to 26.5). 3 patients suffered from controlled arterial hypertension, 3 patients had controlled dyslipidemia, 3 patients were obese and 4 subjects reported smoking habit. Bath-PUVA therapy was performed using 8-methoxypsoralen (8-MOP) dissolved in warm bath, three to four times per week for 5–6 weeks. Patients were immersed in bath containing 0.0001% 8-MOP for 20 min before UVA irradiation. The initial dose was 0.2–0.5 J/cm^2 or 30% of minimal phototoxic dose (MPD) that was gradually increased by maximum of 0.5 J/cm^2 or 30% MPD every other session depending on skin response (RH). Arterial hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of antihypertensive medication. Subjects who reported smoking at least one cigarette per day during the last year were classified as smokers. Hypercholesterolemia was defined as elevated level of total or/and low-density lipoprotein (LDL) cholesterol or use of lipid lowering therapy. According to WHO, the obesity was defined as a body mass index (BMI) ≥30.

2.2. Methods

15 ml of venous peripheral blood was taken from each patient before initiation of therapy and after the last irradiation. Peripheral blood mononuclear cells (PBMCs) were isolated immediately using Ficoll gradient density centrifugation (Biocoll Separating Solution®, BioScience, Cat No. L6113). Total RNA (1 μg) extracted from PBMCs using TRIzol (Invitrogen, Darmstadt, Germany) was reversely transcribed using First Strand cDNA Synthesis Kit for RT-PCR (Roche, Cat No. 04896866001) according to manufacturer's instruction. Expression of selected gene encoding pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells (PBMCs) of patients with psoriasis before and after treatment was assessed with quantitative real-time reverse transcription-PCR. Complementary DNA of interest was detected and quantified by the standard LightCycler® Carousel-Based System (Roche) and normalized to the housekeeping gene-(h-PBGD) (LightCycler® h-PBGD Housekeeping Gene Set, Roche, Cat. No. 03146073001). Primers and probes were purchased from Roche (Table 1). Resulting CT values were normalized using the delta CT method [20]. All analyses were performed in duplicate.

3. Statistical Analysis

Statistical analysis was performed using Graph Pad prism version (system Windows XP). The mean, median, maximal, minimal and standard deviations of values were calculated. The Wilcoxon matched pairs test was used to compare the expression of cytokines in PBMCs before and after NB-UVB and bath-PUVA. Relations between continuous variables of interest were assessed by Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$.

4. Results

NB-UVB therapy resulted in a significant reduction of PASI score ($p < 0.001$). Following NB-UVB therapy, mean PASI score ± SD decreased from 12.6 ± 2.9 to 3.6 ± 0.97 (ranging from 1.1 to 4.7). 14 patients reached PASI 50 (88.2%) whereas 3 patients achieved PASI 75 (23.5%). Bath-PUVA therapy resulted in significant PASI improvement ($p < 0.001$); the mean PASI score ± SD decreased after therapy from 16.5 ± 6.6 to 4.2 ± 2.9 (ranged from 1.2 to 8.5). 4 patients reached PASI 50 and 3 patients achieved PASI 75.The expressions of genes encoding selected cytokines in PBMCs before and after NB-UVB and bath-PUVA therapy in relation to expression of housekeeping gene (h-PBGD) are presented in Figs. 1 and 2, respectively. NB-UVB therapy led to the significant decrease in the expression of IL-17A, TNF-α, and IL-6 mRNA in unstimulated PBMCs, freshly isolated from patients with

Table 1
Sequences of primers.

Target gene	Primers
IL-17A	F5′-TGGGAAGACCTCATTGGTGT-3′
	R5′-GGATTTCGTGGGATTGTGAT-3′
TNF-α	F 5′-GCTGCTCACCTCATTGGAG-3′
	R5′-CCAGGAGAGAATTGTTGCTCA-3′
IL-6	F5′-CAGGAGCCCAGCTATGAACT-3′
	R5′-AGCAGGCAACACCAGGAG-3′
IL-1β	F5′-AAAGCTTGGTGATGTCTGGTC-3′
	R5′-GGACATGGAGAACACCACTTG-3′
INF-γ	F5′-GGCATTTTGAAGAATTGGAAAG-3′
	R5′-TTTGGATGCTCTGGTCATCTT-3′
IL-23R	F5′-CCATCTCTACAGGGCACCTTAC-3′
	R5′-CGATCATTCCCAATAAAAGTCC-3′
IL-10	F5′-TGCCTTCAGCAGAGTGAAGA-3′
	R5′-GCAACCCAGGTAACCCTTAAA-3′