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Photodynamic therapy in psoriasis: suppression of cytokine production in vitro and recording of fluorescence modification during treatment in vivo

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Abstract. Photodynamic therapy (PDT) consists of the combination of photosensitizers absorbing light mainly in the red spectral region and irradiation with light of corresponding wavelengths. We analysed its effects on the cytokine secretion (IL-1β, TNFα, IL-6) of freshly isolated peripheral mononuclear cells from six patients with chronic plaque-stage psoriasis in comparison with PUVA. PUVA treatment resulted in a decreased production of all three cytokines, but most pronounced in the case of IL-6. PDT caused a similar change in the cytokine pattern, but its effectiveness was lower. In vivo fluorescence recordings were performed on psoriatic plaque lesions after topical application of the photosensitizer Photosan-3. Under irradiation, progressive photobleaching was noted with increasing radiation dosage. This is the first reported study of photochemical reactions using on-line fluorescence recordings during PDT of psoriatic lesions in vivo. Our results demonstrate the capacity of PDT to cause immunomodulatory effects similar to PUVA, thus indicating its potential application to the treatment of this common disease.

Key words: Haematoporphyrins – Photochemotherapy – Cytokines – PUVA – Photodynamic therapy – Psoriasis

The administration of photosensitizing psoralens and subsequent irradiation with UVA (PUVA) is a well-established photochemotherapeutic regimen (Parrish et al. 1974). This approach has been proven to be effective in a variety of dermatoses including psoriasis (Gupta and Anderson 1987). In contrast, photodynamic therapy (PDT) combines the administration of photosensitizers absorbing light in the visible spectral region, e.g. haematoporphyrin derivatives (Hpd), and subsequent irradiation with light of the corresponding wavelength around 630 nm (Dougherty 1986). The photosensitizing porphyrin components accumulate preferentially in tumours rather than in normal tissues (Kostron et al. 1986). Tumour irradiation with visible light results in the formation of singlet oxygen which in turn causes tumour regression due to necrosis (Weishaupt et al. 1976). PDT using the Hpd Photofrin-II as photosensitizing agent has been successfully applied to the treatment of a variety of solid tumours, including skin cancer (McCaughan 1990).

Psoriasis is a common inflammatory skin disease exhibiting a complex alteration of the skin immune system (Bos 1988). Initial lesions are characterized by a mononuclear cell infiltrate localized in the upper dermis and only minimal epidermal changes (Ragaz and Ackermann 1979). Mononuclear cells have the capacity to release a variety of cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF), which are involved in the pathogenesis of inflammation (Johnston 1988).

It has recently been shown that PUVA treatment of peripheral blood mononuclear cells of psoriatics results in a decreased release of TNFα and IL-1β, which might contribute to its anti-inflammatory effect (Neuner et al. 1992). The present study showed that similar alterations in the cytokine secretion pattern can also be induced by PDT. Moreover, for the first time we demonstrated the in vivo accumulation of Hpd in psoriatic lesions and photobleaching of this photosensitizer after irradiation with light of wavelength 630 nm indicating the presence of photochemical reactions.

Material and methods

Patients

Six patients with chronic plaque-stage psoriasis were included in the study after written consent was obtained. All of them had severe psoriasis affecting more than 25% of their body surface, but otherwise were in good physical condition.

Isolation of mononuclear cells

Peripheral blood was collected into heparinized syringes. Peripheral mononuclear cells were obtained by Ficoll density gradient sedimentation (Sigma, Deisenhofen, Germany) and cultured at 37 °C in an atmosphere containing 5% CO_2. The medium used was supplemented RPMI-1640 (Seromed, Munich, Germany).

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Photosensitizers

The photosensitizers 8-methoxypsoralen (8-MOP) and the Hpd Photosan-3 were purchased from Sigma and Seelab (Deisenhofen, Germany), respectively. Photosan-3 is a mixture of different prophyrins inclusing haematoporphyrin IX, protoporphyrin IX and deuteroporphyrin IX. In aqueous solution, these porphyrins are present as monomers, dimers, or higher aggregates. 8-MOP was dissolved in 96% ethanol. Photosan-3 and dissolved 8-MOP were diluted in medium before adding to cell cultures.

Light sources

Red light of wavelength 630 nm was emitted by an argon ion pumped dye laser. The power density of irradiation was adjusted to 100 mW/cm^2. The UVA sources were 14 F15T8 Sylvania lamps (Waldmann, Villingen-Schwenningen, Germany) emitting radiation at 315–400 nm with a peak at 365 nm (7 mW/cm^2). UVA doses were controlled with a UV meter (Waldmann). The 407 nm line of a krypton ion laser served as excitation source for fluorescence measurements.

Irradiation protocol

All experiments were carried out in triplicate. Cells were cultured at a density of 10^5/ml in 96-well U-bottom microtitre plates (Nunc, Roskilde, Denmark) in the presence of either 10 μg/ml 8-MOP or a similar concentration of Photosan-3 for 4 h followed by a single exposure to UVA (1–10 J/cm^2) or visible light at 630 nm. Supernatants were harvested 24 h later and stored at −20 °C until the cytokines were measured. Cell viability was determined by trypan blue staining. Except during irradiation, the plates were wrapped in aluminium foil to exclude additional light exposure.

Determination of TNFα, IL-1β and IL-6 in culture supernatants

TNFα and IL-1β were determined with one-step enzyme immunoassays (ELISAs) as described elsewhere (Elsässer-Beile et al. 1993). Briefly, microtitre plates were coated with monoclonal mouse anti-human TNFα or polyclonal goat anti-human IL-1β antibodies. Diluted culture supernatants or tested standards were added to the test wells together with peroxidase-conjugated rabbit anti-human TNFα antibodies or peroxidase-conjugated F(ab')$_2$ fragments of goat anti-human IL-1β antibodies, respectively (reagents kindly donated by Dr. H. Gallati; Hoffmann La Roche, Basel, Switzerland). The specificity of the antibody binding was confirmed by the addition of an excess of unconjugated antibodies. The ELISA sensitivities were 10 pg/ml for the TNFα assays and 25 pg/ml for the IL-1β assay. IL-6 was determined with a commercial immunoradiometric assay (IRMA; Medgenix Diagnostics, Brussels, Belgium). Median cytokine concentrations were determined from triplicates and used for the definition of the median of all six patients. Results were expressed relative to the non-irradiated controls in the absence of photosensitizers (= 100%).

In vivo and in vitro fluorescence recording

Two additional patients with chronic plaque-stage psoriasis were selected for in vivo fluorescence recording. An ointment containing 3.3 mg/ml Photosan-3 was applied topically to a psoriatic lesion 18 and 6 h prior to irradiation. The treated area was then covered by a bandage until irradiation. Fluorescence spectra were recorded from the pretreated site was well as 1 cm from its margin. Fluorescence was detected using a sensor for in vivo measurements and a polychromator combined with an optical multichannel analyser as previously described (König et al. 1990). These recordings were repeated after 20, 40 and 60 s of irradiation (= 2, 4 and 6 J/cm^2) with light at 630 nm. For control purposes, in vitro fluorescence recordings of Photosan-3 in aqueous solution (3.3 μg/ml) were performed using the same detection equipment.

Results

Effects of PUVA treatment on cytokine secretion pattern

Supernatants derived from cells treated with PUVA showed a dose-dependent decrease in cytokine secretion (Fig. 1). At 3 J/cm^2 the concentration of TNFα was reduced to 55%, IL-1β to 38% and IL-6 to 20% of their initial values. Five of six patients showed parallel changes in the cytokine pattern. One patient exhibited an increase in IL-1β concentration from 21% at 3 J/cm^2 to 39% at 10 J/cm^2 (data not shown).

To analyse the influence of both PUVA components we also measured cytokine secretion in response to 8-MOP and UVA separately. Incubation with 10 μg/ml 8-MOP did not reduce the secretion of TNFα (Table 1). Irradiation with 10 J/cm^2 UVA resulted in a decrease to 64%, whereas the complete PUVA protocol yielded a reduction to 38%. Comparable observations were also made for IL-1β and IL-6 (data not shown). This result can partially be explained by the cytotoxic effect of UVA radiation shown by a reduction in viable cells capable of trypan blue exclusion to 83% (Table 1).

Effects of PDT on cytokine secretion pattern

TNFα, IL-1β and IL-6 were also found to be reduced in the supernatants in a dose-dependent manner after PDT (Fig. 2). The reduction in TNFα secretion was greater than that resulting from PUVA treatment: 1, 3 and 10 J/cm^2 reduced TNFα to 79%, 34% and 21% of its initial value, respectively (in contrast to 82%, 55% and 38% in the PUVA protocol). IL-1β secretion was not reduced dramatically by increasing doses of red radiation: 1, 3 and 10 J/cm^2 reduced IL-1β to 76%, 72% and 68% of its initial value, respectively. The reduction in IL-6 secretion was intermediate between that of TNFα and IL-1β: 3 and 10 J/cm^2 reduced IL-6 to 56% and 44% of

Table 1. TNFα secretion and cell viability of peripheral mononuclear cells in response to the components of PUVA and PDT (untreated cells = 100%)

	cell viability (5)	TNFα secretion (%)
8-MOP (10 μg/ml)	96	107
UVA (10 J/cm^2)	81	64
PUVA	75	38
Photosan-3 (10 μg/ml)	97	88
630 nm (10 J/cm^2)	95	79
PDT	94	21

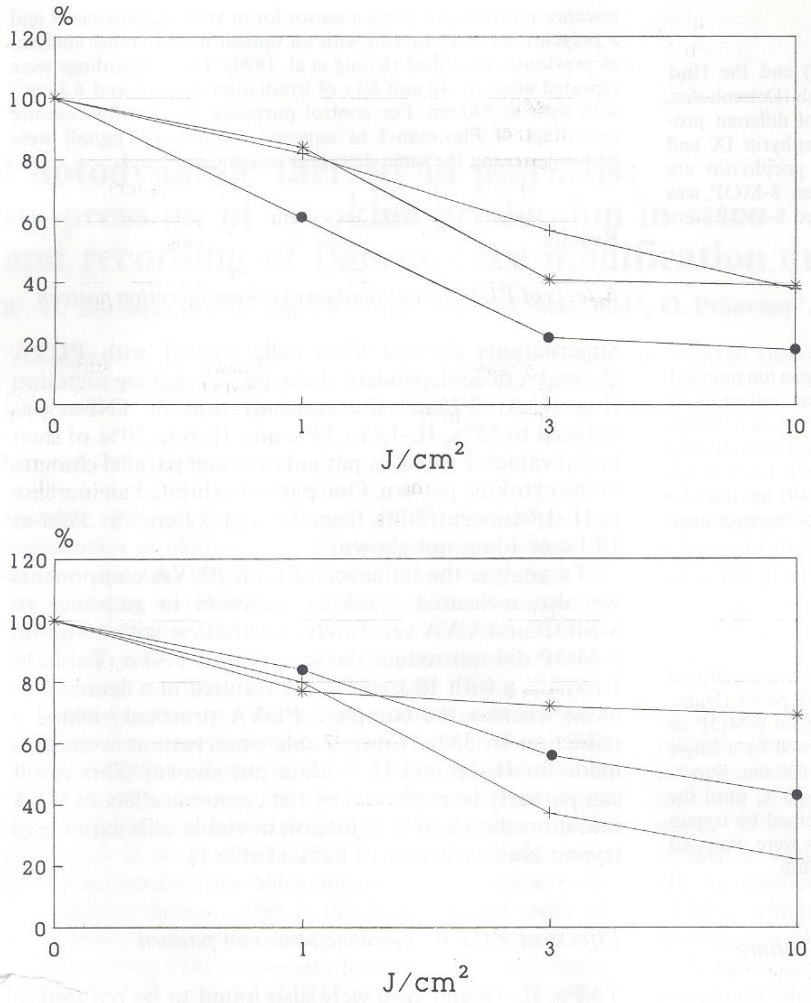

Fig. 1. Alteration in the cytokine secretion pattern of peripheral mononuclear cells from psoriatics following in vitro PUVA treatment (100% = no irradiation and absence of photosensitizer). ●, IL-6; +, TNFα; ∗, IL-β

Fig. 2. Alteration in the cytokine sectretion pattern of peripheral mononuclear cells from psoriatics following in vitro PDT treatment (100% = no irradiation and absence of photosensitizer). ●, IL-6; +, TNFα; ∗, IL-β

its initial value, respectively (in contrast to 15% with 10 J/cm² UVA in the PUVA protocol).

Photosan-3 and light of wavelength 630 nm separately had little influence on cytokine secretion and only marginally altered cell viability (Table 1, data for IL-1β and IL-6 not shown).

Photobleaching during PDT of psoriatic lesions in vivo

To determine whether photoreactions occur in psoriatic lesions treated topically with Photosan-3 we performed on-line fluorescence recordings during PDT of two patients. Fluorescence, and therefore the accumulation of the photosensitizer, was found to be restricted strictly to the area to which the agent had been applied; no fluorescence was detectable 1 cm away from the site of application (data not shown). Subsequently, light of 630 nm emitted by a dye laser was administered and fluorescence was determined after 20, 40 and 60 s of exposure (= 2, 4 and 6 J/cm²). These recordings showed a decrease in fluorescence intensity with increasing dosage (Fig. 3). This result was attributed to the process of photobleaching which involves the photo- and oxygen-induced reaction of the porphyrin photosensitizer (König et al. 1992).

Localization of photochemical reactions

To exclude the possibility that the photochemical reactions observed occurred only at the surface of the skin

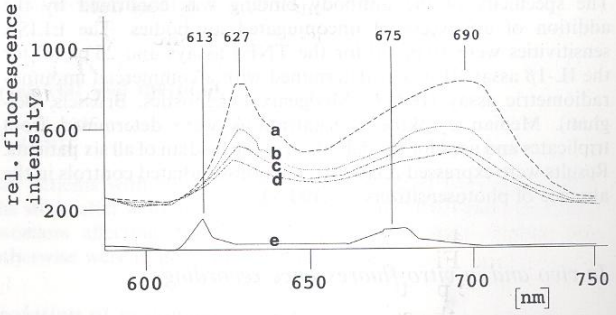

Fig. 3. Fluorescence recording in vitro and in a psoriatic lesion prior to and under irradiation with light at 630 nm wavelength. Note the decreasing fluorescence intensity under irradiation ('photobleaching') and the qualitative differences between the in vitro and in vivo spectra. *a* prior to irradiation; *b* 2 J/cm²; *c* 4 J/cm²; *d* 6 J/cm²; *e* in vitro

and not in the lesional tissue, fluorescence was also determined for Photosan-3 in vitro. The fluorescence detected was markedly lower compared with the in vivo values. More importantly, the fluorescence spectrum also showed qualitative differences (Fig. 3): maxima were observed at 613 nm and 675 nm in vitro and at 627 nm and 690 nm in vivo. Thus, the photoreactions do indeed take place within the lesions.

Discussion

The mechanisms of action of PUVA therapy and of PDT are not fully understood. When psoralens are exposed to UVA radiation two independent photoreactions take place resulting in covalent bonding to one or both strands of DNA (Pathak 1984). This interaction together with the use of UVA radiation for excitation keeps alive the debate about the carcinogenicity of PUVA therapy. In contrast, PDT uses light in the long-wavelength region of the visible spectrum. The site of damage caused by PDT with Photosan-3 is not the nucleus and depends on the length of time of exposure: damage occurs either to the membranes in cases of short exposure or to the mitochondria in cases of prolonged treatment (Kessel 1986). Thus, from a theoretical point of view, the use of PDT seems to be preferred, since the risk of inducing malignancies can be avoided.

Our observations demonstrate the ability of PDT to reduce the secretion of cytokines involved in the pathogenesis of inflammation thus indicating the potential of this therapy in the treatment of psoriasis. It needs to be stressed that at similar concentrations of the photosensitizers and corresponding irradiation doses the effect is not as pronounced as in the case of PUVA therapy with regard to IL-1β and IL-6. The difference, however, can partially be explained by the cytotoxic effects of UVA radiation. Additionally, two other circumstances are likely to augment the in vivo effectiveness of PDT when compared with PUVA. Hpd accumulate in target tissues. The direct injection of haematoporphyrin into subcutaneous tumours has been shown to result in a 44-fold greater concentration in the tumour than in adjacent skin (Kostron et al. 1986). Moreover, light at 630 nm emitted by dye lasers, which are usually used as the light source for PDT, penetrates into tissues much more deeply than UVA radiation. Thus, in vivo application PDT might prove to be superior to PUVA therapy since higher concentrations of photosensitizer could be reached in the target tissue and photoreactions could occur much more deeply within the target lesions.

Two common side effects of PDT are cutaneous photosensitization and systemic immunosuppression (Musser and Fiel 1991). These severe side effects can be avoided if the photosensitizer is applied topically, which is possible in the case of cutaneous lesions. Our in vivo recordings demonstrate that the accumulation of the photosensitizer is indeed strictly limited to the target. Thus, local PDT might be a very safe treatment regimen, not carrying the burden of the severe side effects observed in the case of systemic PDT.

To summarize, we have shown PDT to alter the cytokine secretion pattern of mononuclear cells in patients with psoriasis in a manner similar to PUVA. Photosensitizer fluorescence measured in vivo and on-line during treatment in psoriatic lesions demonstrates both the occurrence of photochemical reactions under irradiation in vivo and a strict limitation of these reactions to the target area. The lower effectiveness of PDT in comparison with PUVA in vitro might be compensated for by the accumulation of Hpd in lesions to be treated and by the better tissue penetration of light of longer wavelengths.

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