In vitro and In vivo Effects of Photodynamic Therapy in Cutaneous T Cell Lymphoma

WOLF-HENNING BOEHNCKE, KARSTEN KÖNIG, ANGELIKA RÜCK, ROLAND KAUFMANN and WOLFRAM STERRY

Department of Dermatology and Institute for Laser Technologies in Medicine, University of Ulm, Ulm, Germany

Photodynamic therapy consists of the combination of photosensitizers absorbing light in the visible spectral region and irradiation with light of corresponding wavelengths. We analysed its effects in comparison to PUVA treatment on cell lines MyLa and HuTT89, established from patients with cutaneous T cell lymphomas. Proliferation was reduced to 50% by exposure to 7.5 J/cm² UV-A (≈ Edₐ). This effect was increased more than 10-fold in the presence of 10 μg/ml 8-methoxypsoralen. The EDₐ for photodynamic therapy using 630 nm light emitted by a dye laser and 10 μg/ml Photosan-3 was found to be about 1 J/cm². In vivo fluorescence recordings during topical photodynamic therapy of mycosis fungoides lesions showed photo-bleaching and thus documented the triggering of photochemical reactions. Our observations document the capability of photodynamic therapy to inhibit proliferation of transformed T cells similar to PUVA, pointing out its potential in the treatment of cutaneous T cell lymphomas.

(Accepted December 1, 1993.)


W.-H. Boehncke, Department of Dermatology, University of Ulm, Oberer Eselsberg 40, D-89081 Ulm, Germany.

PUVA, combining the administration of psoralens as photosensitizers and irradiation with UV-A light (1), is a well established treatment for a variety of immunological diseases affecting the skin (2). In contrast, photodynamic therapy (PDT) consists of systemic intravenous application of photosensitizers absorbing light of the visible spectral region, e.g. hematoporphyrin derivatives, and subsequent irradiation with light of the corresponding wavelength around 630 nm (3). The photosensitizing porphyrin components accumulate preferentially in tumours rather than in normal tissues (4–6). Irradiation results in the formation of singlet oxygen, which in turn causes tumour regression due to necrosis (7, 8). This regimen has been successfully applied in the treatment of a variety of solid tumours including skin cancer (9–11).

Cutaneous T cell lymphomas are lymphoproliferative malignancies mainly of CD4+ T cells (12). PUVA is one of several well established and evaluated therapeutic approaches (13). However, its mechanism of action in this disease is still not fully understood, since responses were reported also in lesions too thick to be completely penetrated by UV-A light. Höningnann et al. (14) speculate that cells from deeper layers move upward once superficial tumour cells are destroyed, reaching a level where UV-A light can effectively penetrate.

Besides PUVA, PDT has also been reported to be effective in the treatment of mycosis fungoides (15, 16). We therefore investigated the effects of PDT on proliferation, morphology, and distribution of photosensitizers on a T cell line established from a patient with mycosis fungoides. These observations were compared to results obtained with PUVA therapy.

Material and Methods

Cell lines

T cell line MyLa was established from a patient with mycosis fungoides as described elsewhere (17, 18). Cell line HuTT89 (19), derived from the peripheral blood of a patient with Sézary’s syndrome, was purchased from the American Type Culture Collection.

Photosensitizers

8-methoxypsoralen (8-MOP) and hematoporphyrin derivate Photosan-3, comparable to Photofrin II, were purchased from Sigma (Germany) and Seelab (Germany), respectively. Photosan-3 is a mixture of different porphyrins like hematoporphyrin IX, protoporphyrin IX, deuteroporphyrin IX. In aqueous solution, these porphyrins are present as monomers, dimers, or higher aggregates. Eight-MOP was dissolved in 96% ethanol. Photosan-3 and dissolved 8-MOP were diluted in medium prior to adding to cell cultures.

Light sources

630 nm red light was emitted by an argon-ion laser pumped dye laser (100 mW/cm²). The UV-A sources were 14 F15T8 Sylvania lamps (Waldmann, Germany) emitting light of 315–400 nm with a peak at 365 nm (7 mW/cm²). UV-A doses were controlled with a UV meter (Waldmann, Germany). The 407 nm line of a krypton-ion laser served as excitation source for fluorescence measurements.

Cell proliferation

To determine the effects of PUVA therapy and PDT on proliferation, 2×10⁵ cells per well were placed in U-bottom microtitre plates. Eight-MOP and Photosan-3 were added to a final concentration of 10 μg/ml, and the plates were then wrapped in aluminum foil and kept in the dark to prevent uncontrolled light exposure. After 6 h of incubation, cells were washed 3 times prior to and after a single exposure to either UV-A light or 630 nm light. In parallel, cell cultures not incubated with any photosensitizer were similarly treated. During the final 6 h of a 24-h culture period, 1-H-thymidine was added and the uptake was determined. Experiments were performed in triplicates; results are shown in percent proliferation with spontaneous proliferation set to 100%.

Fluorescence pattern and morphological changes

To visualize the distribution of the photosensitizer within the cells we used video-intensified fluorescence microscopy with a highly sensitive silicon intensified target camera (Hamamatsu, Japan) as described elsewhere (20). The same camera was used to determine morphological changes of the cells detected by phase contrast microscopy. In the case of Photosan-3 the fluorescence intensity in the spectral range 590–800 nm was detected after excitation with the 405/436 nm bands of a 50 W mercury, high pressure lamp. The fluorescence intensity of 8-MOP was detected in the spectral range 520–550 nm after excitation with the UV band (356 nm). Power densities of less than 100 mW/cm² were applied to limit photochemical modifications during the detection time.

In vivo fluorescence recording

Plaque lesions in two patients with mycosis fungoides were selected for in vivo fluorescence recording. An ointment containing 3.3 mg/ml
PDT exhibits an inhibitory effect comparable to PUVA

Neither light of 630 nm wavelength (Fig. 2) nor incubation with 10 µg/ml Photosan-3 (data not shown) affected proliferation of MyLa or HuT78. However, irradiation of cells pre-incubated with Photosan-3 over 6 h in the dark resulted in proliferation inhibition. At about 1 J/cm² inhibition was half maximal (Fig. 2). Again, data obtained from cell line MyLa were similar to the results in the case of HuT78.

Eight-MOP shows increasing fluorescence intensity under irradiation

To analyse the cellular distribution pattern prior to and after irradiation, video-intensified fluorescence microscopy was performed on cell line MyLa. By means of this technique, a diffuse distribution of 8-MOP was detected within the cytoplasm before irradiation (Fig. 3a). Exposure to UV-A light resulted in a profound increase of fluorescence throughout the cell, no longer sparing the nucleus (Fig. 3b). This effect was paralleled by morphological changes: a marked swelling of the cell occurred, the regular shape was lost, and vacuoles appeared within the cytoplasm (Fig. 3c, 3d).

Irradiation of cells incubated with Photosan-3 results in photobleaching

MyLa cells incubated with Photosan-3 exhibited a fluorescence pattern similar to 8-MOP before irradiation (Fig. 4a). Here, light exposure caused a gradual decrease of fluorescence throughout the cytoplasm (data not shown). This phenomenon is known as "photobleaching". The morphological changes observed were similar to those described for 8-MOP (Fig. 4b, 4c).

Photobleaching during PDT of mycosis fungoides lesions in vivo

To determine whether photoreactions occur at all in cutaneous T cell lymphoma lesions treated topically with Photosan-3, we performed on-line fluorescence recordings during PDT of plaque lesions in two patients with mycosis fungoides. Fluorescence as a means to determine the accumulation of the photosensitizing agent was found to be strictly restricted to the area to which this agent was applied; no fluorescence was detectable 1 cm aside of its application (data not shown). Subsequently, light of 630 nm emitted by a dye laser was administered and fluorescence was determined every 10 s for 40 s of exposure (= 0.11, 0.22, 0.33 and 0.44 J/cm²). These recordings showed a decrease in fluorescence intensity with increasing dosage (Fig. 5). This process of "photobleaching" documents the photo- and oxygen-induced reaction of the porphyrin photosensitizer (21).

Photochemical reactions take place in the tissue rather than at the skin surface

To exclude the possibility that the photochemical reactions observed occurred only at the surface of the skin but not in the lesional tissue, fluorescence was also determined for Photosan-3 in vitro. The fluorescence detected was markedly lower compared to the in vivo values. More importantly, the fluorescence spectrum also showed qualitative differences (Fig. 5); two
maxima were observed at 613 nm and 675 nm, respectively. In contrast, the maxima in vivo were at 630 nm and 691 nm. Thus, the photoreactions documented do take place within the lesions.

DISCUSSION

The mechanisms of action are not fully understood in either PUVA or PDT. When psoralens are exposed to UV-A light two independent photoreactions take place, resulting in covalent binding to one or both strands of DNA (22). This interaction, together with the use of UV-A light for excitation, keeps the discussion about the potential risk to induce cancer by PUVA therapy alive. In contrast, the site of damage caused by PDT with Photosan-3 is not the nucleus. Damage occurs at membranes in case of short exposure, and the mitochondria are altered in case of long treatment (23). Thus, the use of PDT seems to be favourable from a theoretical point of view, since the risk of inducing malignancies can be avoided.

Our observations document the ability of PDT to inhibit proliferation of malignant transformed T cells, pointing out the potential of this regimen in the treatment of cutaneous T cell lymphomas. It needs to be stressed that at similar concentrations of the photosensitizers higher doses of irradiation are needed in the case of PDT to obtain comparable inhibition of proliferation.

Fig. 3. Fluorescence increase of 8-MOP. Distribution of 8-MOP in MyLu before (a) and after (b) excitation with light of 356 nm and morphological changes (c and d) are documented.

Fig. 4. "Photobleaching" of Photosan-3. Distribution of Photosan-3 in MyLu prior to irradiation (a) and morphological changes caused by excitation with light of 405/436 nm (b and c) are recorded.
PDT in comparison to PUVA in vitro might be compensated by
the accumulation of hematoporphyrin derivatives in lesions to
be treated and by the better tissue penetration of light with
longer wavelengths.

ACKNOWLEDGEMENT

This study has been supported by grant P.122 of the University of Ulm.

REFERENCES

1. Parrish IA, Fitzpatrick TB, Tanebaum L, Pathak MA. Photo-
chemotherapy of psoriasis with oral methoxsalen and long-wave


3. Dougherty TJ. Photosensitizers: therapy and detection of malignant

4. Kostron H, Bellnier DA, Lin CW, Swartz MR, Mattuza RL. Distribu-
tion, retention and photoactivity of hematoporphyrin derivate

5. Bellnier DA, Dougherty TJ. The time course of cutaneous por-
phyrin photosensitization in the murine ear. Photochem Photobiol

DR. Photodynamic therapy of murine skin tumors using Photo-

oxygen as the cytotoxic agent in the photoinactivation of a murine

8. Parker JG. Optical monitoring of single oxygen generation during
photodynamic treatment of tumors. IEEE Circuits Devices Mag
1987, January, 10–21.

9. McCaughan JS. An overview of experiences with photodynamic
therapy for malignancies in 192 patients. Photochem Photobiol

10. Wilson BD, Mang TS, Cooper M, Stoll H. Use of photodynamic
therapy for the treatment of extensive basal cell carcinomas. Facial

11. McCaughan JS. Photodynamic therapy of skin and esophageal

12. Steery W. Systemic lymphomas with skin infiltration. In: Fitz-
patrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. 4th
Hill Book Company, in press.

13. Powell FC, Spiegel GT, Muller SA. Treatment of parapsoriasis and
mucosal porphyrins: the role of psoralen and long-wave ultraviolet

Photopherotherapy of cutaneous T cell lymphomas. J Am Acad

15. Dougherty TJ. Annual report to the U.S. Food and Drug Admini-
stration, IND 12, 678.

Photodynamic therapy with topical 8-aminolevulinic acid for treat-
ment of cutaneous carcinomas and cutaneous T cell lymphoma.

17. Kalloto K, Bischole S, Rasmussen HF, Thesmop Pedersen K,
Thomsen K, Steery W. A continuous T-cell line from a patient with

18. Kalloto K, Bishole S, Dyrberg T, Boel E, Rasmussen PB, Thesmop
Pedersen K. Establishment of two continuous T-cell strains from
a single plaque of a patient with mycosis fungoides. In Vitro Cell

Cutaneous T cell lymphoma and leukemia cell lines produce
and respond to T cell growth factor. J Exp Med 1981; 154:
1403–1418.
distribution of photosensitizing porphyrins measured by video-
photodynamic activity of endogenous protoporphyrin in human
24. Musser DA, Fiel RJ. Cutaneous photosensitizing and immuno-
suppressive effects of a series of tumor localizing porphyrins.
Competition between photobleaching and fluorescence increase of
photosensitizing porphyrins and tetralsulfonated chloroaluminium-
Fluorescence formation during PDT in the nucleoli of cells incu-
bated with cationic and anionic water-soluble sensizers. J