Review Article

Photodynamic Therapy of Skin Cancers: Sensitizers, Clinical Studies and Future Directives

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Photodynamic therapy (PDT) is a new modality of skin cancer treatment. It involves the administration of photosensitizing drugs which, when localized in tumor tissue can produce its destruction by absorbing an adequate dose of light of an appropriate wavelength. A large number of photosensitizing agents have been tested in PDT experiments. Topical application of 5-aminolevulinic acid (5-ALA) followed by light irradiation is the most commonly used method. 5-ALA is a prodrug converted *in situ* via the heme cycle into protoporphyrin IX, an effective photosensitizer agent. Treatment of nonmelanoma skin cancers by PDT has met with varying degrees of success. In the case of 5-ALA, this therapy's main limitation is the poor penetration of 5-ALA into skin, due to hydrophilic and charge characteristics. However, the efficacy of 5-ALA-PDT may be improved by (a) development of adequate drug delivery systems; (b) use of enhancers of PpIX production and accumulation in target tissue, and (c) modifications of the 5-ALA molecule. Optimal timing, light sources, doses, and number of applications are also important factors for topical 5-ALA therapy and must be well defined. The aim of this review is to highlight recent progress in 5-ALA-PDT of skin cancer, and to present ways holding promise for its improvement.

KEY WORDS: photodynamic therapy; 5-aminolevulinic acid; photosensitizer; protoporphyrin IX; skin cancer.

INTRODUCTION

The concept of photochemical sensitization resulting in specific or nonspecific cell death was first put into oncological use in 1903, when eosin and light were combined to treat skin cancer (1). However, sensitizers like eosin or acridine orange are no longer in use because of their toxicity and carcinogenicity. The first systematic clinical study of photodynamic therapy (PDT) for malignant lesions was initiated in 1977 (2); it led to an abrupt increase in interest on PDT (3).

In PDT, three components are combined to induce tumor destruction: a sensitizer, light, and oxygen. An amplifying description of PDT follows: administration of a photosensitizer or its precursor by different routes (topical, oral, intravascular, or local intratumor injection) followed by a time interval permitting photosensitizer distribution, localization, and accumulation in tumors. Malignant lesions are then exposed to light of a wavelength appropriate for photosensitizer absorption. Photoactivation of the dye results in the formation of reactive oxygen species of which singlet oxygen ($^1O_2$) is reported to be the major species primarily responsible for the ensuing cytotoxicity (4).

A wide variety of malignancies involving various organ systems—head and neck, central nervous, lung, ocular, esophageal, gastrointestinal, genitourinary, and gynecologic—have been treated with PDT. However, this therapy has shown its most promising results in dermatology, due to the accessibility of skin to light exposure. Many centers in Canada, United States, Japan, and Europe now recognize PDT as a useful therapeutic modality.

Nonmelanoma skin cancers, which include basal and squamous cell carcinomas, represent the most common malignant neoplasms in humans (5). Traditionally, these tumors are treated by surgery, electrodesiccation, cryosurgery, topical application of podophyllin or 5-fluorouracil, and radiotherapy. These forms of therapy are costly, can leave a scar and hypo- or hyperpigmentation, and may have other side effects such as pain, severe inflammation, irritation, and crusting which may last for weeks (6–9). The search for more acceptable types of therapy has led to proposals for alternative forms of treatment, leading PDT to become a first option for therapy of several cutaneous diseases (10).

Kalka et al. (11), in a recent review, described the extensive application of PDT in dermatology, including gains in the understanding of mechanisms of tumor destruction. The aim of the present review is primarily to familiarize readers with PDT by describing the principles of this innovative procedure, the main classes of sensitizers, their mechanisms of action, and parameters to be considered in the choice of appropriate light irradiation. Previous information (11) is supple-

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ABBREVIATIONS: 5-ALA, 5-aminolevulinic acid; AK, actinic keratoses; BCC, basal cell carcinoma; BD, Bowen's Disease; CR, complete response; DMSO, dimethylsulfoxide; EDTA, edetic acid disodium salt; o/w, oil-in-water; PDT, photodynamic therapy; PpIX, protoporphyrin IX; SCC, squamous cell carcinoma; $^1O_2$, singlet oxygen.

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mented by highlighting recent clinical progress in topical PDT of skin cancer using 5-aminolevulinic acid (5-ALA) as well as promising new approaches in the field.

PHOTOSENSITIZERS USED IN PDT

A large number of photosensitizing agents have been tested *in vivo* and *in vitro* in PDT experiments, but none has shown ideal properties. Prerequisites for an ideal sensitizer include chemical purity, selectivity for tumoral cells, chemical and physical stability, a short time interval required between administration of the sensitizer and its maximal accumulation in tumor tissues, activation at wavelengths with optimal tissue penetration, and rapid clearance from the body (1,12).

The main classes of photosensitizers are porphyrin derivatives, chlorins, phthalocyanines, and porphycenes (12).

Porphyrin

The most extensively used photosensitizers are porphyrin derivatives. Hematoporphyrin derivative (HPD) is the first systematically studied (13) photosensitizer used for clinical PDT. Its purified form, porfimer sodium (Photofrin II®) is, in many countries, the only photosensitizer approved for clinical PDT of bladder, lung, esophageal, gastric, and cervical cancer (11). The porphines, $TPPS_4$ for example, are synthetic porphyrins, which have potential usefulness for topical treatment of skin tumors (14).

Chlorins

Chlorins are reduced porphyrins with a strong absorption band in the 640–700 nm range (1). The chlorin group includes derivatives from chlorophyll or porphyrins: mono-L-aspartyl chlorin e6 (15) and diaspartyl chlorin e6 (16). Benzoporphyrin derivative monoacid ring A (BPD-MA) has been found to be effective in treating basal cell and squamous cell carcinomas (11,12).

Phthalocyanines

Phthalocyanines are second-generation photosensitizers, containing a diamagnetic metal ion, which have shown high photodynamic efficiency in the treatment of animal tumors, as well as reduced phototoxic side effects. Aluminum disulfonated phthalocyanines are chemically stable, readily soluble in water, and have a strong absorption in the red part of the spectrum at 675 nm (17). Chloroaluminum-tetrasulfophthalocyanine (ALPcTS) has been used in clinical studies of basal cell carcinomas, Kaposi's, sarcoma and lung cancer (11).

Porphycenes

Porphycenes, synthetic porphyrins, and isomers of porphines, are efficient generators of singlet oxygen and of tumor regression (12). 9-acetoxy-2,7,12,17-tetrakis-(-methoxyethyl)-porphycene (ATMPn) is a chemically pure substance with fast pharmacokinetics and superior photodynamic properties *in vitro* and *in vivo* as compared to Photofrin II® (18).

Other Photosensitizers

Hypericin, an active plant pigment, has been suggested as a new photosensitizer for photodynamic therapy. It shows maximum absorption in the ultraviolet (330 nm) and visible (550 and 588 nm) light range, and phototoxic effects *in vitro* and *in vivo* (19,20). Lutetium texaphyrin (Lu-Tex) is water soluble and has the additional advantage of strong absorption near 730 nm. It shows good effects on regression of tumors in mice (21). Hypocrellins (22), methylene blue, azure C, methylene violet, thionine, methylene green, hematoporphyrin, Nile blue A (23), and rhodamine 123 (24) are potential photosensitizers for PDT.

Systemically administered photosensitizers like hematoporphyrin and its more purified form Photofrin®-II, when used in clinical trials, presented relatively slow rates of clearance from the skin, causing generalized skin photosensitivity persisting for up to 8–10 weeks after treatment (3,25–27). Such serious side effects intensified the investigation of topical application of sensitizers in dermatology for the last 10 years.

5-ALA generates endogenous porphyrins, in particular, protoporphyrin IX (PpIX), via the heme cycle (28). Its topical application represents at present, the most commonly used PDT technique in dermatology. Considering its importance and wide application, 5-ALA will be discussed in detail in this review.

MECHANISM OF ACTION OF PHOTOSENSITIZERS

The mechanisms of cellular destruction induced by the combination of a photosensitizer and light irradiation include several reactions initiated by the absorption of light photons by the sensitizer, causing it to be promoted from ground to the excited singlet state. It then either decays to the ground state or undergoes intersystem crossing to the triplet excited state: in this process the spin of an excited electron is reversed, and a change in multiplicity of the molecule results. The excited sensitizer can react with surrounding oxygen molecules in two ways. *Type I* photoxidation involves direct reaction with a substrate by a mechanism mediated by hydrogen or electron transfer, yielding radicals that may react with oxygen to form free oxygen radicals, initiating radical chain reactions. *Type II* pathway involves energy transfer from the excited sensitizer to nearby molecular oxygen to produce $^1O_2$, which initiates oxidation of susceptible substrates. Type I and II pathways occur simultaneously and competitively. Type II reaction, however, appears to play the central role in cytotoxicity, because of the highly efficient interaction of the $^1O_2$ species with various biomolecules. Singlet oxygen is believed to be the main cytotoxic agent in PDT (1,11,29).

Tumor cell destruction is mediated by cellular necrosis and/or apoptosis (30). In addition to direct damage to neoplastic cells, vascular damage plays an important role in tumor eradication. In PDT, oxygen radicals induce changes in both tumor and surrounding normal vasculature, decreasing the barrier function of endothelial cells, depriving neoplastic cells of nutrients (1,3,31). Because some aspects of its cytotoxicity have not been elucidated, the mechanisms direct or indirectly involved on cell death by PDT remain the subject of intense investigation.

LIGHT SOURCES, WAVELENGTHS AND DOSES FOR PDT

Different light sources have been used in clinical and experimental PDT. Lasers that emit light at specific wave-

lengths (630 nm) as well as incoherent light sources such as slide projectors with polychromatic spectra were found to be effective for dermatological application of 5-ALA-based PDT (12,32).

Laser systems are widely used for treatment of dermatological conditions. The gold vapor laser (628 nm), the argon ion-pumped dye laser (630 or 635 nm), and the copper vapor-pumped dye laser constitute the most popular systems. These systems permit the selection of a wavelength that has a maximal effective tissue penetration of approximately 10 mm, and have been used in combination with all types of sensitizer agents. The laser beams can be launched into an optical fiber applicator, enabling light to be delivered directly into internal tumors. However, these techniques are relatively expensive, require specialized supporting staff and are space-consuming (3,12,33). It is probable that such systems will eventually be substituted by laser diode arrays which are very convenient by being easily moved, requiring only a single phase supply and also being relatively inexpensive (3,11).

Commercially available incoherent light sources, such as incandescent or arc lamps, have been used in topical 5-ALA-based PDT by several groups mainly for treatment of large lesions (12). Because coherence of light is not necessary for PDT, such sources offer the advantage of being less expensive and easier to handle (3). The most popular of them is the filtered slide projector, which excludes light below 600 nm with glass filters, minimizing the emission of shorter wavelengths, which by being strongly absorbed by hemoglobin, could lead to the production of heat-induced erythema (34). Despite this, unfiltered white light has also been employed, and recently, professional incoherent lamp like PDT 1200 lamp (Waldmann) has been developed for PDT (35).

The light source for PDT must exhibit suitable spectral characteristics coincident with the absorption maximum wavelength range of the sensitizer applied. Thus, for 5-ALA to induce PpIX for PDT, it seems that a coherent light source at 635 nm is more effective than irradiation at 630 nm (36). Incoherent light sources emitting both 635 and 670 nm radiations could improve the efficacy of PDT by the formation of photoproducts of PpIX that can be excited at about 670 nm (37).

The light doses used in PDT are given in $J \cdot cm^{-2}$, which is a result of the multiplication: energy fluence rate of the source ($W \cdot cm^{-2}$) × radiation time (seconds) (38). Several studies on 5-ALA-based PDT, including clinical trials and use of animal models, have applied a total light dose varying within the wide range of 10–250 $J \cdot cm^{-2}$ for laser systems, and 30–540 $J \cdot cm^{-2}$ for incoherent sources.

USE OF 5-ALA FOR TOPICAL PDT

A new approach to PDT based on the use of endogenous porphyrins was introduced in 1990 (39). It made use of 5-ALA, which in contrast to porphyrins is a small, soluble molecule able to penetrate the abnormal stratum corneum overlying skin tumors.

5-ALA as such, is not a photosensitizer. It is formed *in vivo* in mitochondria by the condensation of glycine and succinyl CoA, catalyzed by ALA-syntase, becoming the first intermediate for the biosynthesis of heme (Fig. 1). After several further reactions, PpIX is formed and converted to heme by ferrochelatase in the presence of iron. Free heme can inhibit the synthesis of ALA by a negative feedback mechanism (32,40). An excess of exogenous ALA applied topically passes rapidly through an abnormal epidermis and bypassing feedback inhibition, is converted within mitochondria to PpIX, which may accumulate due to the limited capacity of ferrochelatase to convert it to heme (40,41). The conversion of PpIX into heme in cells is known (28) to be a relatively slow process; note that it has been reported that accumulation of PpIX is greater in malignant than in normal tissue (42,43). Porphobilinogen deaminase, the enzyme that converts 4 porphobilinogen molecules into protoporphyrin precursor, uroporphyrinogen III, may have a regulatory role in 5-ALA-induced increase of PpIX. Porphobilinogen deaminase could be a rate-limiting determinant for the amount of PpIX synthesized when 5-ALA is administered in PDT (44,45).

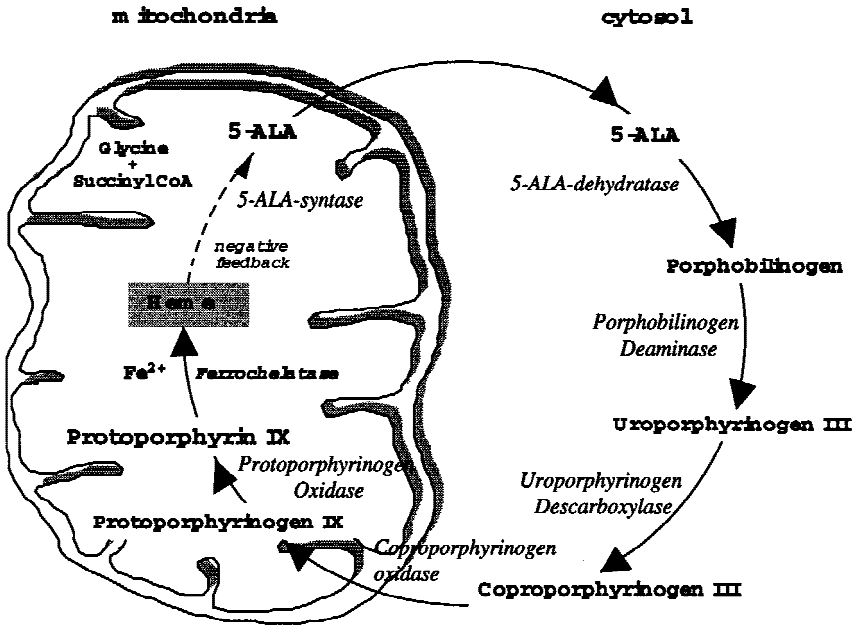

Fig. 1. Heme cycle. The biosynthesis of heme occurs partly in mitochondria and partly in cytosol.

PpIX is a cyclic tetrapyrrole, the immediate precursor of various hemes and chlorophylls, having unique photo-optical properties, leading to the emission of an intense red fluorescence when excited by light (32). Tissues that accumulate a sufficiently high concentration of PpIX may show phototoxic damage after adequate exposure to light (40). For tumor treatment, the absorption peak of the longest visible wavelength ($\lambda \approx 635$ nm) is usually used for excitation, due to good tissue penetration. Following excitation, some of the PpIX molecules present are activated to the triplet state by intersystem crossing. The presence of paramagnetic species such as molecular oxygen enhances intersystem crossing, consequently decreasing fluorescence. By transferring energy from the triplet state of a PpIX molecule to a nearby oxygen molecule, $^1O_2$ will be generated (46). Biological damage may also be caused by other reactive oxygen species such as superoxide anions and hydroxyl radicals, which also arise from $^1O_2$ (29).

5-ALA-induced PpIX fluorescence shows a certain degree of tissue specificity, which may have different causes. A strong correlation between intensity and localization of ALA-induced PpIX fluorescence and the intensity and localization of subsequent phototoxic damage to the skin has been demonstrated. A few hours following an intraperitoneal injection of ALA, sebaceous glands and hair follicles developed very strong PpIX fluorescence, while the basal layer of the epidermis and the dermis showed, respectively, much weaker and essentially no fluorescence (40).

Following synthesis in mitochondria, 5-ALA-induced PpIX selectively accumulates in this organelle and some investigators have indicated that the primary cause of cell death, after PDT, is mitochondrial phototoxicity (47–49).

Studies in human volunteers and experimental animals have shown that 5-ALA-induced PpIX is almost completely cleared from the body within 24 h. Such rapid clearance lowers the risk of PpIX accumulation leading to prolonged photosensitivity, even when PDT treatment is repeated as often as every other day (32).

Topical 5-ALA-PDT presents several potential advantages over more traditional forms of therapy: it is noninvasive, has a short photosensitization period, can be used to treat multiple lesions by short treatment sessions, produces excellent cosmetic results by not causing damage to surrounding skin, has no side effects beyond slight pain during irradiation, and is well accepted by patients (41).

CLINICAL STUDIES WITH 5-ALA-PDT IN DERMATOLOGY

Several investigators have examined topical 5-ALA-PDT for superficial skin cancer including actinic keratosis (AK), basal cell carcinoma (BCC), squamous cell carcinomas (SCC), and Bowen's disease (BD); degree of success ranged from 50% to almost 100%. The differences in response ratios are probably due to differences in treatment protocols, including 5-ALA vehicles, length of application, light sources, wavelengths, doses, and criteria for patient selection. Topical therapy seems to be less effective on thick lesions, or lesions covered by a layer of normal epidermis (41,50).

The first clinical studies with 5-ALA (39) involved topical application of 20% 5-ALA in a oil-in-water (o/w) emulsion; after 3–6 h, 80 BCC, 10 AK, and 6 SCC were exposed to light from a slide projector equipped with a 500-W lamp. The total dose of light varied from 15 to 150 $mW \cdot cm^{-2}$. Ninety percent of the BCC and AK, and 100% of the SCC showed complete response 2–3 months following treatment. In 1992, the same group demonstrated a regression rate of 79% for more than 300 superficial BCCs (28). Table 1 summarizes the results of 5-ALA-based PDT obtained on clinical trials performed according to different protocols. "Complete response" (CR) refers to lesions showing complete clearance, with no clinical or histopathologic signs after the follow-up period (in months).

Wolf et al. (25) treated 13 patients with 70 lesions on the face, scalp, or trunk, including 37 superficial BCC, 6 SCC, 9 AK, and 10 noduloulcerative BCC with an o/w emulsion of 20% 5-ALA for 4–8 h. The light source was a slide projector equipped with a filtered 250-W lamp. A total dose of 30–100 $J \cdot cm^{-2}$ was applied. The authors observed a CR in >80% of superficial BCC, SCC, and AK. Only one of all noduloulcerative BCC treated showed CR.

Svanberg et al. (51) exposed 21 patients with 55 superficial BCC and 25 nodular BCC, 3 patients with 10 BD, and 2 patients with 4 lesions of cutaneous T-cell lymphoma to a pulsed frequency-doubled neodymium laser pumping system at 630 nm and a dose of 60 $J \cdot cm^{-2}$, 4–6 h after treatment with 20% 5-ALA in an o/w emulsion. A CR of >90% was observed for superficial BCC and BD; 64% for nodular BCC, and 50% for T-cell lymphomas.

Using 20% 5-ALA in a cream base for 6–8 hours and an argon-pumped dye laser, at 630 nm (doses in the range 60–80 $J \cdot cm^{-2}$), Calzavara-Pinton et al. (33) treated different skin cancers in 85 patients, observing CR in 100% of 6 lesions of BD and 4 keratoacanthomas, 84% in 50 AK, 86.9% in 23 BCC, 50% in 30 nodular BCC, 83.3% in 12 SCC, and 33.3% in 6 lesions of nodular SCC after 24–36 months. Four pigmented BCC cases tested were resistant to topical PDT.

Szeimies et al. (8) applied an o/w emulsion containing 10% 5-ALA to 10 patients with 36 lesions of AK. After 6 h, the lesions were exposed to an incoherent light device: PDT 1200 (Waldmann, Germany), emitting at 580–740 nm with a total light dose of 150 $J \cdot cm^{-2}$. A CR in 71% for the cases of AK localized on the head was observed. None of AK located on the hands and arms showed complete remission.

Morton et al. (52) compared the effectiveness of 5-ALA-based PDT with cryotherapy for the treatment of 40 BD. An o/w emulsion containing 20% 5-ALA was applied for 4 h; the irradiation source was a prototype lamp with a filtered 300-W xenon short arc plasma discharge (~630 nm). The lesions received a dose of 125 $J \cdot cm^{-2}$. The cryotherapy protocol produced complete response in 10 out of 20 cases following single treatment. 5-ALA-based PDT resulted in clearance of 15 out of 20 cases after one treatment, and of all remaining 5 lesions after a second treatment; this treatment led to lesser side effects. After 12 months, CR of 90% in the cryotherapy group and of 100% in the PDT group, respectively, were observed.

Jeffes et al. (53) treated 40 patients with 240 lesions of AK with an emollient vehicle containing 5-ALA at 10, 20, and 30%, respectively. After 3 h, the lesions were exposed to argon pumped dye laser (630 nm) with light doses of 10–150 $J \cdot cm^{-2}$. After 8 weeks there was a CR in 91% of face and scalp AK lesions treated with 30% 5-ALA, and 45% for AK at trunk and extremities.

Wennberg et al., (9) treated BCC, nodular BCC, and SCC for 3 h with an o/w emulsion containing 20% 5-ALA.

Table 1. Summary of Some Studies Results on the Topical Use of 5-ALA-PDT for the Treatment of Primary Skin Cancers Published Since 1990

References	5-ALA formulation	Timing (h)	Light source and λ	Light doses ($J \cdot cm^{-2}$)	Skin lesions (n)	CR (%)	Follow-up (months)
39	20% in o/w emulsion	3–6	Tungsten, >600 nm	31.5–540	80 BCC	90	2–3
					8 SCC	100	2–3
					10 AK	90	18
28	20% in o/w emulsion	3–6	Tungsten, >600 nm	31.5–540	>300 BCC	79	3
25	20% in o/w emulsion	4–8	Tungsten unfiltered or >570 nm	30–100	37 sBCC	97	3–12
					6 SCC	83	3–12
					9 AK	100	3–12
51	20% in o/w emulsion	4–6	Neodymium: Yag-dye laser system, 630 nm	60	55 sBCC	100	<14
					25 nBCC	64	6–14
					10 BD	90	6–14
33	20% in o/w emulsion	6–8	Argon ion-dye laser, 630 nm	60–80	23 BCC	87	24–36
					12 SCC	84	24–36
					6 BD	100	24–36
					50 AK	100	24–36
8	10% in o/w emulsion	6	PDT 1200 (incoherent) 580–740 nm	150	36 AK	71	3
52	20% in o/w emulsion	4	Xenon short arc discharge lamp ~630 nm	125	20 BD	100	12
9	20% in o/w emulsion	3	Xenon lamp, 620–670 nm	75–100	190 BCC	92	6
					10 nBCC	20	
					18 SCC	61.1	
53	10, 20, or 30% in emollient vehicle	3	Argon ion-dye laser, 630 nm	10–150	240 AK	91	3
54	10% in a nanocolloid lotion	6	Halogen unfiltered	240	55 BCC	85	6

Note: o/w, oil-in-water; sBCC, superficial basal cell carcinoma; nBCC, nodular basal cell carcinoma; AK, actinic keratosis; BD, Bowen's disease; λ, wavelength.

Irradiation was performed using a filtered (620–670 nm) incoherent source (ILC 402 equipped with a CERMAX 300-W xenon lamp). The doses were 75 or 100 $J \cdot cm^{-2}$. A CR ratio of 92% for 190 lesions of BCC, 20% for 10 nodular BCC, and 61.1% for 18 SCC lesions was observed after 6 months.

5-ALA was encapsulated in nanoparticles, which were processed to a nanocolloid lotion containing 10% of the active substance. Hürlimann et al. (54) applied this formulation to 55 lesions of BCC in 19 patients under an occlusive dressing for 6 h prior to irradiation with visible light from an unfiltered 250-W halogen lamp (total light dose: 240 $J \cdot cm^{-2}$). After a single treatment, 85% of BCC lesions showed a CR after a follow-up time of at least 6 months.

Little information is available on the efficacy of topical 5-ALA-PDT for treatment of malignant melanoma. The high pigmentation of melanoma tissues may limit the efficiency of this treatment by inhibiting light penetration (13,25,55). Psoriasis is an example of the application of topical 5-ALA-PDT to a nontumoral condition. The accumulation of PpIX in areas of plaque psoriasis in 15 patients presenting a total of 42 plaques has been described (56). The results obtained show that the characteristic fluorescence emission of PpIX increased within the 6-h period following application of 5-ALA, suggesting a potential use of superficial PDT in such cases. The variability of the fluorescence intensity was as great between plaques at different sites on the same patient as that between different patients.

Several authors (8,28,53) have reported that in 5-ALA-based PDT, following therapeutic application of light, patients experience within a few seconds an irritation described as "itching" or "burning." Application of a local anesthetic can reduce the discomfort caused by this histamine-like reaction (52,57), considered as more of a nuisance than a hazard (28).

FUTURE DIRECTIVES FOR PDT

New Sensitizers and Delivery Vehicles

The development of new photosensitizers, presenting more affinity for neoplastic cells and shorter periods of sensitization, is at present a matter of intense investigation in the PDT field. Recently, new sensitizers for PDT were reported (58). These compounds, namely 21-thiaporphyrin, 21,23-dithiaporphyrin, and 21-oxaporphyrin, reveal some of the properties required for such therapy. They showed physicochemical, chemical, and pharmacological features which indicate their potential as sensitizers to be applied in clinical PDT.

Several strategies have been adopted to increase the selectivity of photosensitizers for target tissues, including the use of delivery systems, which should carry the sensitizer preferentially to tumor cells. Lipid-based delivery vehicles, such as liposomes and water-in-oil emulsions for example, allow the systemic administration of zinc-phthalocyanine, purpurins, porphycenes, hematoporphyrin, etc. Plasma lipoproteins can transport photosensitizers, such as the benzoporphyrin derivative monoacid ring A, to various areas of the body, including tumor tissues; this interaction is mainly governed by hydrophilicity/ lipophilicity of the sensitizer. Chlorin e6 monoethylenediamine monoamide can be linked to monoclonal antibodies, which recognize specific antigens of tumors cells. This monoclonal antibody-photosensitizer conjugates have shown good selectivity in tumor cells *in vitro. In vivo* studies are not conclusive (59).

Another example for the use of liposomes as delivery vehicle was recently described. Hypocrellin A, a novel sensitizer of high singlet oxygen quantum yield, was administrated as an intravenous injection of a unilamellar liposome yielding a high, vehicle-dependent retention in mice sarcomas (60).

Improvement of 5-ALA-PDT

At present, 5-ALA seems to be the most frequently used topical agent for PDT. 5-ALA is a hydrophilic molecule and a zwitterion at physiological pH. Hydrophilic charge compounds present limited capacity to cross biological barriers such as the stratum corneum of the skin. This capacity, being a fundamental condition for 5-ALA conversion into PpIX (61), indicates that the major limitation of topical 5-ALA-PDT may reside in the extent of 5-ALA penetrability into the stratum corneum of the epidermis.

Improved delivery systems of 5-ALA to the skin may play an important role in the success of PDT. Enhancement of skin penetration of drugs can be achieved either by varying vehicle composition in order to increase partition of drugs into the skin, or by altering their skin permeability (62,63).

Penetration Enhancer, Iron Chelators, Liposomes, Nanocolloid Lotion and 5-ALA Derivatives

In vitro experiments have shown that treatment of cells with chemical compounds such as dimethylsulphoxide (DMSO), edetic acid disodium salt (EDTA), desferrioxamine, or allyl-isopropylacetamide can enhance 5-ALA-induced PpIX formation by interference with the heme cycle (64).

DMSO is also a promoter of malignant cell differentiation, which at certain concentrations can induce enzymes of the heme cycle (65). In addition, DMSO acts as a potent penetration enhancer for several drugs into skin (66). EDTA is an iron chelator believed to cause inhibition of ferrochelatase activity (67).

Our personal experience showed that the presence of 20% of DMSO in o/w emulsions increased the *in vitro* permeation of 5-ALA through hairless mouse skin. *In vivo* studies demonstrated significant increase of the amount of PpIX extracted from healthy hairless mouse skin after 3 h of treatment. By confocal scanning laser microscopy, an observed increase in red fluorescence in skin that had received this treatment was attributed to PpIX (68).

Consistent with *in vitro* and *in vivo* findings using animal models (68,69), clinical studies have demonstrated significant regression of different types of human tumors (Harth 1998; Soler 1999). Administration of o/w cream containing 20% 5-ALA, 2% DMSO, and 2% EDTA for 12 h (70) or pretreatment with 99% DMSO followed by the administration during 3 h of 20% 5-ALA and 2% DMSO mixed in a cream base (71) and subsequent irradiation of the tumors with incoherent light sources, at doses of about 100 $J \cdot cm^{-2}$, were utilized in both studies.

Desferrioxamine is an iron chelator that appears to compete with ferrochelatase for ferric ions, preventing heme formation. Topical PDT using a combination of 5-ALA and 3% desferrioxamine has been shown to be effective in the treatment of superficial BCC, nodular BCC, solar keratosis and BD (72).

Liposomes are of great scientific and medical interest due their ability to protect and carry hydrophilic and/or hydrophobic molecules (73). Topical administration of drugs encapsulated in liposomes leads to extensive and more selective accumulation of these substances in skin. The possibility of endogenously synthesizing porphyrins in tumor cells by using free 5-ALA or encapsulated in liposomes was examined by *in vitro* addition of encapsulated 5-ALA to tumor, liver, skin, kidney, and brain explants from tumor-bearing mice. The study indicated that PpIX formation was enhanced when compared to results using nonencapsulated 5-ALA. *In vivo* studies, using the same model, confirmed these findings (74,75). Our preliminary *in vitro* experiments have demonstrated that the encapsulation of 5-ALA in liposomes, having a lipidic composition similar to the human stratum corneum, provided a higher retention of 5-ALA into the full thickness of hairless mouse skin. This suggests that one strategy to increase the rate of penetration of 5-ALA into the skin could be its encapsulation in lipid vesicles. However, the efficiency of encapsulation, the stability of 5-ALA-containing liposomes, its skin permeation profile, and retention remain to be better defined.

Vehicles that provide sustained delivery of 5-ALA such as a nanocolloid lotion, showed promise for the treatment of BCC (54), as described before (see Table 1). A more detailed investigation, comparing the effectiveness of this formulation with that of, for example, an o/w emulsion is necessary, as are studies on 5-ALA stability and its delivery profile from nanoparticule preparations.

Another way to overcome the limited bioavailability of 5-ALA may be the use of a prodrug. A prodrug is a pharmacologically inactive precursor of a drug, the spontaneous or enzymatic transformation of which within the body leads to the release of the active drug. Prodrugs usually have improved delivery properties relative to the drug proper. Lipophilic 5-ALA prodrugs, such as 5-ALA ester derivatives, are expected to cross cellular membranes more easily than 5-ALA; after entering the site of action, 5-ALA esters are converted enzymatically to 5-ALA, which in its turn, is converted into PpIX (76). Several 5-ALA ester derivatives have been synthesized with C1–C8 aliphatic alcohol side chains. Their use in different cell cultures has been shown to result in PpIX levels higher than those produced by 5-ALA itself (61,76–78). However, further investigation of the potentialities and toxicity of 5-ALA ester derivatives in humans, as well as systematical clinical studies, remain to be done.

Physical Methods

Skin permeation of drugs can be increased by physical methods such as iontophoresis and ultrasound. Iontophoresis involves the delivery of small, charged molecules into the skin by application of an electrical current. Positive or negative drug ions act as charge carriers across the high-impedance stratum corneum, causing rapid delivery of the drug to the epidermis. The method is painless and noninvasive, and consistent amounts of the drug are delivered (79). The water-soluble and ionic nature of 5-ALA makes it adequate for iontophoresis, and a system for rapid and quantifiable topical 5-ALA delivery has been developed (80). 5-ALA was iontophoresed from a 2% solution in sterile deionized water into the skin of upper inner arm of 13 healthy volunteers at

charges varying from 3 to 120 mC. PpIX accumulation was detected. Under optimal conditions, iontophoresis could provide a rapid method for topical delivery of 5-ALA. Such optimization is at present being studied (81).

Ultrasound has been used to increase transdermal permeation of drugs in the treatment of cancer. Balb/c nude mice bearing tumors (human colon adenocarcinoma cell line) implanted in their flanks, received a 20% 5-ALA cream topically on the tumors 10 min prior to or immediately after ultrasound (1 MHz system, energy intensity 3 $W \cdot cm^{-2}$ in a continuous or pulsed mode) (82). Enhanced production and homogenous distribution of PpIX were observed at the tumor sites after treatment. Further studies are necessary to determine the limitations of ultrasound in 5-ALA-PDT, which appears to be, at a first glance, a promising technique for the improvement of 5-ALA penetration in tumors.

Light Devices and Radiation Protocols

The time interval between 5-ALA administration and exposure to light, characterizing the period required for maximal PpIX accumulation, is of great importance. Each of the above-described different 5-ALA delivery methods needs the determination of its optimal timing.

Light dosimetry is closely related to PDT efficacy (32). In the face of many different parameters related to light sources, it is difficult to compare results obtained from clinical studies on 5-ALA-based PDT because no standard protocols for light exposure are available. Differences in coherent or incoherent light sources, wavelength (filter or unfiltered light), energy fluence rate from the source, tumor/light source distances, and doses are found. Furthermore, light doses may have been fractionated by different ways.

The utilization of repeated irradiation aimed at increased PDT efficacy, following a single topical 5-ALA application, has been examined in animal models (83–86). It appears that a second illumination, following a period of darkness for the formation of new PpIX, results in additional cell death, increasing PDT efficacy. It is possible that 5-ALA still present in the treated tissue can be converted into PpIX by the surviving cells (86). The specific design of more potent light sources for PDT, presenting several wavelength options, as well as permitting better management with respect to the anatomy of the target tissue, may improve PDT efficacy.

Influence of Temperature

Recently, studies in human tumor cell lines (87) and in animal models (88) demonstrated the influence of temperature on 5-ALA-induced PpIX formation. Increasing human skin temperature from 31 to 36°C, led to an approximate 50% increase of PpIX fluorescence (87). This temperature dependence was related to PpIX synthesis and not to the penetration of 5-ALA into the skin. Although these experimental studies are promising, the influence of temperature on PpIX formation awaits confirmation for different tumors and for condition prevailing in man.

CONCLUSIONS

PDT is a therapeutic modality for skin cancer treatment that combines photosensitizing agents, light, and oxygen for the destruction of tumors. The photosensitizing agents described up to the present have limited affinity for tumor tissues and may cause skin photosensitivity. Therefore, topical application of photosensitizers for treatment of skin cancer has been the objective of intense investigation over the last decade. Several clinical studies have demonstrated favorable results and minimal side effects following treatment of superficial skin cancer by topical application of the PpIX precursor, 5-ALA. The efficacy of 5-ALA-PDT may be improved by further development of drug delivery systems, use of enhancers of PpIX accumulation, and modifications of the 5-ALA molecule. Better understanding of the mechanisms involved in tumor destruction following PDT is required. More precise determination of optimal timing for topical 5-ALA application; development of simpler, less expensive and more efficient light sources; information on a number of PDT applications in repetitive treatments; and definition of dose fractionation schemes for the different skin lesions encountered in clinical practice would become equally helpful.

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