Chapter 14

Topical Photodynamic Therapy

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Contents

14.1 Introduction

At the beginning of the 20th century Hermann von Tappeiner, director of the Institute of Pharmacology at the University of Munich, coined the term "photodynamic reaction" to describe the oxygen-dependent tissue reaction following photosensitization and irradiation with light [1]. Today it is known that photodynamic therapy (PDT) requires the simultaneous presence of a photosensitizer, light and oxygen in the diseased tissue. The photosensitizer accumulates in the target cells and absorbs light of a certain wavelength. The energy is transferred to oxygen and highly reactive oxygen species (ROS) – mainly singlet oxygen – are generated. Following an appropriate light dose the reactive oxygen species directly lead to cell and tissue damage by inducing necrosis and apoptosis and indirectly stimulate inflammatory cell mediators (Fig. 14.1).

In recent decades, PDT has gained worldwide popularity, first as an experimental therapy for a variety of human cancers. Mainly porphyrins, chlorin derivatives or phthalocyanines have been studied so far for primary and adjuvant cancer therapy [2]. However, for dermatological purposes, only hematoporphyrin derivatives (HPD) such as porfimer sodium (Photofrin) and porphyrin-inducing precursors such as 5-aminolevulinic acid (ALA) and methyl aminolevulinate (MAL) are of practical use. As systemic photosensitizing drugs induce prolonged phototoxicity [3], topical photosensitizers are preferred for use in dermatology. Meanwhile drugs such as ALA and MAL have reached approval status for the treatment of epithelial cancers or their precursors throughout the world and there is growing interest in the use of PDT not only for nonmelanoma skin cancer but also

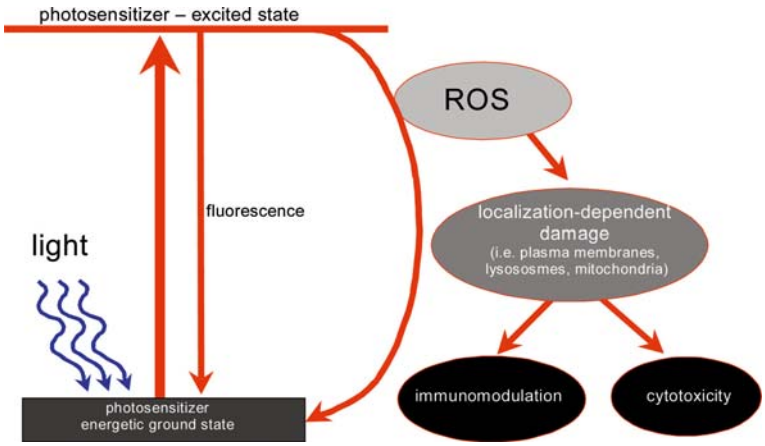

Fig. 14.1. A photosensitizer molecule in the excited state is able to form ROS, mainly singlet oxygen (reaction type II) via photooxidation. Depending on the subcellular location of the dye, organelle-specific damage occurs. The specific damage sites plus the extent of damage lead to immunomodulation and/or cytotoxicity (from Szeimies RM et al. (2001) PDT in Dermatology in: Krutmann J et al. Dermatological Phototherapy and Photodiagnostic Methods, Springer)

for other skin tumors such as lymphoma or for tumor surveillance in transplant patients as well as for non-oncological indications such as psoriasis, localized scleroderma and skin rejuvenation [4–6].

14.2 Mechanism of Action

During irradiation the photosensitizer absorbs light and this is converted to an energetically higher state, the "singlet state". After the short half-life period (approximately 10^{-9} s), the activated photosensitizer returns to the ground state after emission of fluorescence and/or internal conversion, or changes from the singlet state into the more stable triplet state with a longer half-life (10^{-3} s) ("intersystem crossing"). In the type I photooxidative reaction there is direct hydrogen and electron transfer from the photosensitizer in the triplet state to a substrate. This reaction results in the generation of radicals of the substrate. These radicals are able to react directly with molecular oxygen to form peroxides, hydroxy radicals and superoxide anions. This type I reaction is strongly concentration-dependent. Cells can be directly damaged by this reaction, especially when the photosensitizer is bound to easily oxidizable molecules.

In the photooxidative type II reaction, electrons or energy are directly transferred to molecular oxygen in the ground state (triplet) and singlet oxygen is formed. The highly reactive state of singlet oxygen results in a very effective oxidation of biological substrates. Both reaction types can occur in parallel, as substrate and molecular oxygen are present together with the photosensitizer in the triplet state. The type of reaction that preferentially occurs depends on the photosensitizer used, the subcellular location of the dye and the substrate, and the oxygen supply around the activated photosensitizer. Indirect experiments in vitro have indicated that singlet oxygen is the main mediator of PDT-induced biological effects.

Following activation of a photosensitizer with light of the appropriate wavelength ROS, in particular singlet oxygen, are generated. Depending on the amount and location in the target tissue, these ROS modify either cellular functions or induce cell death by necrosis or apoptosis [2, 7] (Fig. 14.2).

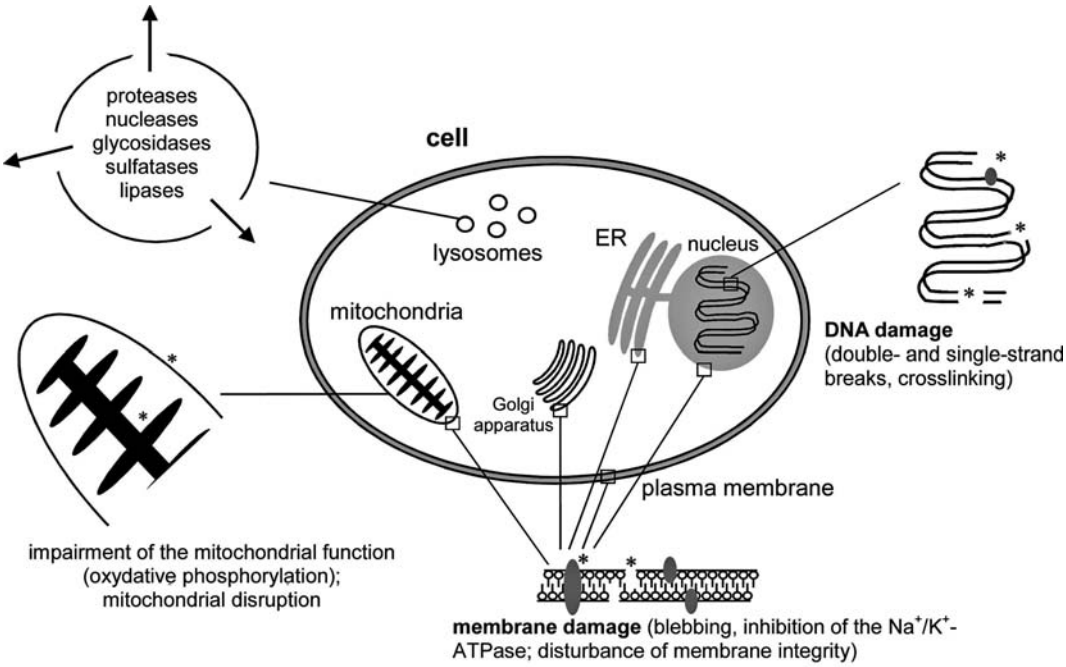

Fig. 14.2. PDT-related subcellular damages depend on the localization of the photosensitizer used. Significant damage occurs mostly at the plasma membranes, lysosomes and mitochondria. This results in disturbance of the membrane integrity, the release of lysosomal enzymes and impairment of the respiratory chain. Damage to the DNA does not contribute significantly to cellular necrosis (from Ref. 7)

14.3 Photosensitizers

Topically applied dyes including eosin red and erythrosine were the first "photosensitizers" used to treat conditions such as pityriasis versicolor, psoriasis, molluscum contagiosum, syphilis, lupus vulgaris and skin cancer [1]. The tumor localizing effects of porphyrins have been studied since 1908. In the late 1970s Dougherty used HPD to treat skin cancer [1, 2], and this resulted in a renewal of interest in PDT. The main problem in the use of HPD is the prolonged skin photosensitization which lasts for several weeks [8]. Topical application of these drugs is not possible since the rather large molecules (tetrapyrrol rings) do not penetrate the skin. Therefore the introduction of porphyrin precursors such as ALA and MAL by Kennedy and coworkers in 1990 was a significant milestone in the development of PDT in dermatology as the small low molecular weight molecules can easily penetrate the epidermis [2]. Currently in Europe MAL is approved under the name of Metvix for the treatment of basal cell carcinoma and actinic keratoses (AK) in combination with red light. It is also registered in the US for AK under the name Metvixia but is not yet marketed. In the U.S., Metvixia® is approved for treating AKs since 2004, while 5-ALA hydrochloride (Levulan® Kerastick™) is approved for photodynamic treatment of AKs in combination with blue light since 1999 [3]. Photosensitisers based on 5-ALA are not photoactive by themselves, but show a preferential intracellular accumulation in the altered cells of the diseased tissue and are metabolized during haem biosynthesis to photosensitizing porphyrins quite selectively in these cells [7, 9]. If no surface illumination is given, the photoactive porphyrins are metabolized to the photodynamically inactive haem within the following 24 to 48 hours [2].

Since proliferating, relatively iron-deficient tumor cells of epithelial origin are highly sensitized by ALA and MAL, tissue damage is mostly

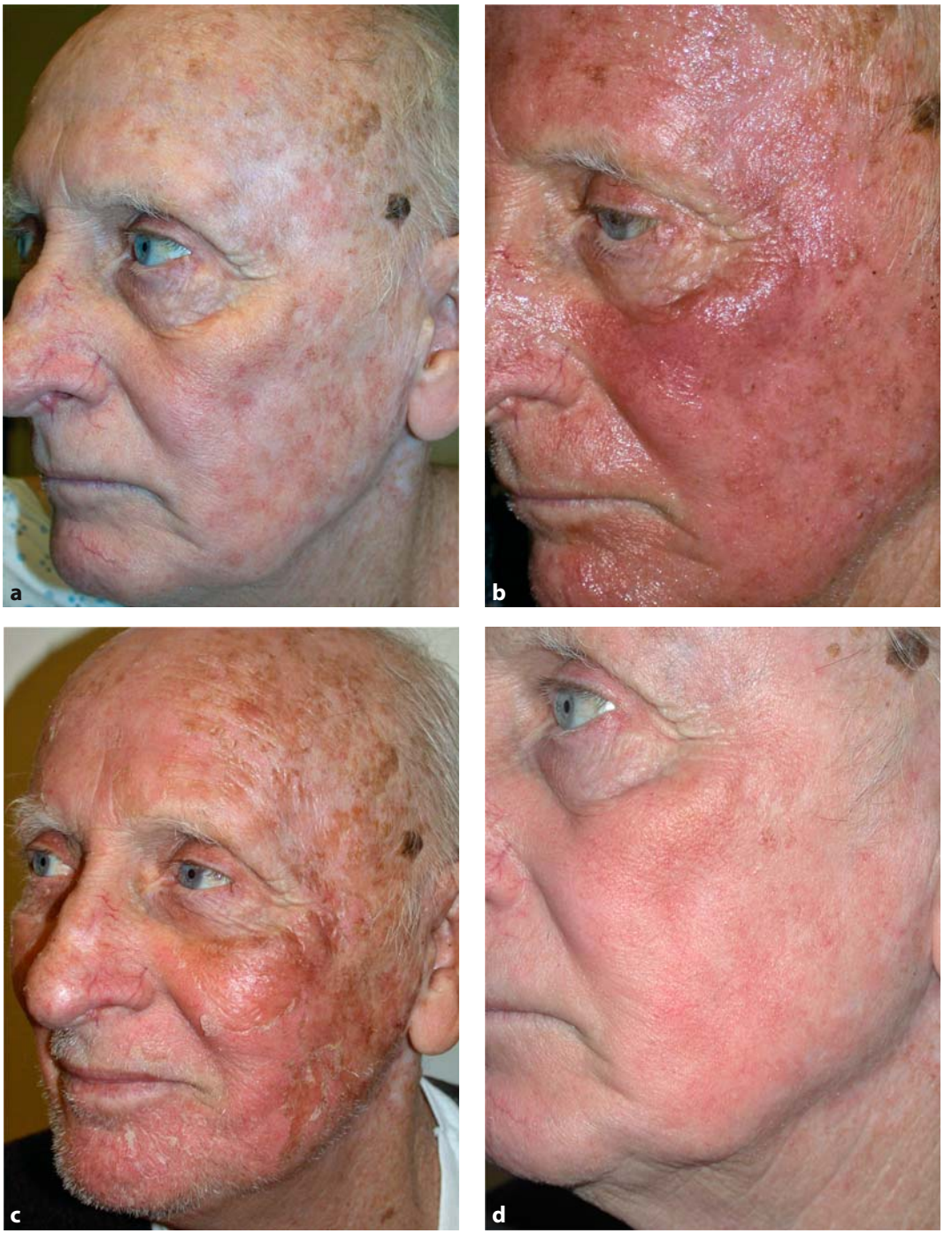

Fig. 14.3. 73-year-old patient who underwent ALA-PDT (Levulan Kerastick) for actinic keratoses: **a** pretreatment; **b** 48 hours after treatment; **c** 1 week after treatment; **d** 1 month after treatment. Note the significant improvement in skin texture and discoloration

restricted to the sensitized cells, almost omitting the surrounding tissue, especially cells of mesenchymal origin such as fibroblasts, resulting in excellent cosmesis (Fig. 14.3) [9]. Aside from two case reports which may be no more than coincidence, no reports on the carcinogenicity of ALA/MAL-PDT have been published [9]. Moreover, in a recent study even long-term topical application of ALA and subsequent irradiation with blue light in a hairless mouse model did not induce skin tumors [10]. Stender et al. have even shown a delay in photoinduced carcinogenesis in mice following repetitive treatments with ALA-PDT [11].

14.4 Light Sources Used in Topical PDT

Protoporphyrin IX (PPIX), the leading porphyrin induced after ALA or MAL sensitization is maximally activated at 409 nm, in the Soret band area of the spectrum, with significantly lower peaks at 509, 544, 584, and 635 nm (Q bands). The light sources used for PDT include red light sources (metal halide, LED, xenon lamps with cut-off filters), argon lasers, simple slide projectors or other broadband light devices, intense pulse light (IPL) lasers, and pulsed dye lasers. Blue light, with a peak wavelength of 417 nm (range 412–422 nm) corresponds to the area of maximal PPIX light absorption, and provides the most effective light activation of PPIX. A device delivering blue light (BLU-U, DUSA Pharmaceuticals, Wilmington, Mass.) has the additional advantage of a large field diameter that allows treatment of broad areas such as the full face and scalp. Blue light PDT has been shown to be effective for nonhypertrophic AK at the low fluence of 10 J/cm², requiring approximately 16 min 40 s of light exposure time. Using this light source with ALA, an incubation time of 14–18 hours is currently the only PDT method approved by the FDA for the treatment of AK. Blue light is believed to penetrate less than 2 mm into skin, and therefore should be restricted in use to the treatment of AK only [12].

Longer wavelengths of light, such as red light, are desirable when deeper parts or thicker lesions as in Bowen's disease or basal cell carcinoma are being targeted, but then higher light doses (usually 75–100 J/cm², depending on the bandwith of the light source) are needed to compensate for the porphyrin's lower absorption coefficient in that wavelength range. IPL, which provides a range of wavelengths of light, and flashlamp-pumped pulsed dye lasers are also suitable for PDT since they emit light in the activating range [13]. The advantages of these light sources over blue light are a greater time efficiency, the possibility of stacking pulses in order to intensify the treatment, and the improvement of associated vascular and pigmented lesions in broad facial treatments. However, they are more expensive and require technical skill.

In summary, blue light is the most potent light source for activation of ALA-induced porphyrins but restricted to superficial lesions. IPL and pulse dye lasers are expensive and technique-sensitive, but they are time-efficient and useful for stacking pulses to thicker individual lesions.

14.5 Topical PDT for Actinic Keratoses

The affinity of topical PDT for dysplastic skin has made it an immediate research tool in the area of AK. Indeed, topical PDT has been shown in several studies to be highly effective for the treatment of AKs. In 1999 the FDA approved topical PDT as safe and efficacious for the spot treatment of AKs as an alternative to liquid nitrogen cryotherapy [14, 15]. The FDA-approved protocol specifies a 14–18-hour delay between 20% δ-ALA application and blue light exposure (see Table 14.1), an incubation period used in a large US study using ALA and blue light in 243 patients with multiple AKs. In this study, 77% and 89% of the patients had 75% or more of the treated lesions cleared at weeks 8 and 12, respectively [16]. Using a similar treatment approach in 36 patients, Jeffes et al. demonstrated an 88% clearance rate of AKs, and showed a lack of correlation between lesional fluorescence and clearance rates [17]. Touma et al. reported that shorter incubation times of 1, 2 and 3 hours were sufficient for a photodynamic reaction to occur [6]. In that study 18 patients with four or more

Table 14.1. Protocol for topical PDT in epithelial cancers and precancerous lesions with different photosensitizers

Photosensitizer	ALA 20% in custom made oil-in-water emulsions or gels	Levulan Kerastick 20% solution	Metvix/Metvixia 16% cream
Application	Occlusive, light impermeable	Occlusive, light impermeable	Occlusive, light impermeable
Incubation time (h)	4–6	14–18	3–4
Light source	Blue, green or red light (indication-dependent)	Blue light (400–450 nm)	Red light from LED source
Irradiation parameters			
Light intensity (mW/cm^2)	100–180	10	Approximately 60
Light dose (J/cm^2)	120–180	10	37
Indication	Epithelial precancerous lesions, superficial basal cell carcinoma	Actinic keratoses	Superficial and nodal basal cell carcinoma and actinic keratoses
Treatments	One (retreatment if needed)	One (retreatment if needed)	Two sessions 7 days apart

AKs had their whole face treated. Thin AKs were reduced by 89.5% at 1 month and these results were maintained at 5 months. There were no differences among the 1-, 2- or 3-hour incubation groups. Szeimies and Pariser applied MAL to AKs for 3 hours followed by 75 J/cm^2 red light illumination and found a 75– 89% resolution of thin lesions, respectively [18, 19]. However, in the trial by Szeimies et al., MAL-PDT was compared to cryotherapy and showed no statistically significant difference [18]. In contrast to the current recommendations, MAL-PDT was only performed once in this trial, whereas a similar study performed in Australia revealed a significantly higher complete remission rate for MAL-PDT (91%) in the treatment of AKs, compared to a single freeze–thaw cycle of cryotherapy (68%) or placebo (30%) [20].

In a study by Ruiz-Rodriguez et al. using 20% ALA to AKs with a 4-hour incubation time followed by full-face IPL treatment with a 615-nm cut-off filter in 17 patients with photodamage [21], there was 91% resolution of AKs after two treatment sessions, with a follow-up of 3 months. Alexiades-Armenakas and Geronemus [22] used the long pulse 595-nm pulse dye laser with fluences of 4–17.5 J/cm^2, after 20% ALA incubation times of 3 or 14–18 hours, to treat patients with AKs of the face and extremities. AK clearance rates were in the 75–90% range at 3 months with no effect of incubation times on clinical response. Topical PDT has been shown to be effective in transplant patients with diffuse AKs [23], as well as in patients with oral leukoplakia [24]. In addition, studies in UV-irradiated mice treated weekly with PDT demonstrated considerable protection from skin cancer, suggesting a potential prophylactic role in photodamaged patients with a high risk of skin cancer [25].

Patients undergoing topical PDT for AKs develop edema, erythema, crusting of AKs and generally mild to moderate discomfort in the first 24–48 hours after PDT. Recovery with desquamation of the treatment areas occurs typically within 7–10 days, depending on the severity of the underlying actinic damage. When compared to other treatment modalities such as liquid nitrogen, 5-FU, imiquimod and medium depth chemical peels, topical PDT offers comparable, if not better, clearance rates of nonhypertrophic AKs [26, 27] while allowing physician-controlled treatment, improved tolerance, and a short recovery time.

In summary, studies have shown that topical PDT clears up to 90% of nonhypertrophic AK. The advantages of PDT over other traditional therapies are its controlled nature, short recovery time, and excellent cosmetic results. Topical PDT is most useful in patients with diffuse AKs.

14.6 Topical PDT for Skin Rejuvenation

One of the main advantages of topical PDT is the excellent cosmetic result seen after the treated lesions have healed, unlike destructive methods that can leave hypopigmentation and scarring. In a study by Goldman, blue light PDT was used to treat 32 patients with incubation times of 15–20 hours [28]. Improvement in skin texture was seen in 72% of treated subjects. In the study by Touma et al. in 18 patients (11 women, 7 men) with AKs and mild to moderate diffuse facial photodamage, using incubation times of 1–3 hours and blue light activation of ALA, there was a statistically significant improvement in overall skin quality, notably sallowness and fine wrinkling. There was limited but noticeable improvement in mottled hyperpigmentation, but this was of borderline statistical significance. Coarse wrinkling seemed to improve in some patients but without reaching statistical significance. Patient satisfaction with the treatment was high. Patients with more severe photodamage appeared to react more intensely to PDT [6].

In the study by Ruiz-Rodriguez et al. using full-face IPL PDT with a 615-nm cut-off filter, and two sessions at an interval of 1 month [21], cosmetic results were excellent, with no pigmentary abnormalities or scarring. Broad facial PDT using IPL and pulsed dye lasers is currently being explored for the purpose of enhanced photorejuvenation. Using ALA to augment IPL and laser treatment of photodamage and vascular lesions is a promising new application, and is also currently being studied. This new indication holds the potential for reducing the need for multiple treatment sessions with these modalities. Microdermabrasion or other methods that ablate the stratum corneum may prove useful if used prior to topical PDT, as they may promote an improved and more rapid penetration of ALA.

Histology of tape-stripped skin treated with ALA PDT and a broadband light activator shows ultrastructural changes limited to the epidermis, with apoptotic damage to keratinocytes within one day of PDT, and relative sparing of melanocytes and Langerhans cells [29, 30]. This explains the improvement in skin appearance seen with topical PDT, and the limited improvement seen in the pigmentary changes of photodamage. The effect of PDT on dermal collagen is still not clear, as some studies have shown neocollagenesis [31], while others have shown that topical PDT induces the collagen-degrading metalloproteinases 1 and 3 together with a reduction in collagen production leading to an antisclerotic effect in patients with scleroderma [32]. Until further studies elucidate PDT's dermal effects, clinical experience appears to support a stimulatory role of collagen in normal skin. An unpublished limited study by Touma comparing topical ALA-PDT with trichloroacetic acid chemical peeling of diffusely actinically damaged skin has shown similar, if not improved, results, as well as neo-collagenesis, with PDT.

In summary, blue light topical PDT penetrates well enough into actinically damaged but otherwise normal skin to improve the texture and fine lines as well as the general appearance of the skin through epidermal renewal. IPL and lasers may be more beneficial for skin rejuvenation because of their depth of penetration, and their ability to treat associated signs of actinic damage, such as lentigines and telangiectasia.

14.7 Fluorescence Diagnosis

Due to the specific accumulation of the ALA-induced porphyrins in tumor cells following either topical or systemic administration, light-induced fluorescence makes the tumor cells visible. Following activation by light of the appropriate wavelength the photosensitizer molecules are excited to a higher energy state and emit during decay fluorescence. Since ROS should not be induced in fluorescence diagnosis significantly lower light intensities (2–5 mW/cm^2) are used as compared to PDT.

Using an optical detection system, e.g. Dyaderm Professional (Biocam, Germany), the induced fluorescence is displayed on a screen under ambient light and the localization and extension of skin tumors can be determined. For fluorescence diagnosis the strong absorption of porphyrins around 400 nm (Soret band) is used,

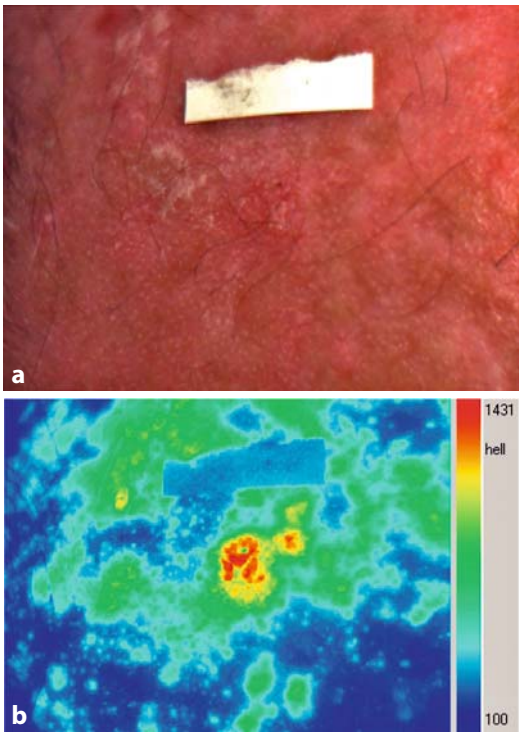

Fig. 14.4. Clinical image (**a**) and false-color coded image (**b**) of an actinic keratosis on the forehead of a 68-year-old-male using fluorescence diagnosis for better delineation. The highest fluorescence intensity (yellow–red color) indicates the margins of the lesion

and thus blue light activates the porphyrins. Because the penetration of blue light is limited to 1 mm, only superficial lesions can be detected, but the method is used very successfully intraoperatively [33].

Fluorescence diagnosis is of particular help in pretreated areas with scars and erythema, where even an experienced dermatologist has difficulty in distinguishing scar from precancerous lesions or skin cancer. Fluorescence diagnosis is an investigator-independent procedure that enables the most appropriate biopsy site to be chosen. Using digital image analysis and reference algorithms the suspected area can be shown in false colors to give maximal contrast between tumor and surrounding tissue (Fig. 14.4). The probability of false-negative biopsies is thus reduced significantly [34].

Another option is fluorescence-guided resection of a skin tumor which minimizes the tissue defect in particular in the skin, and also reduces the number of re-excisions. Image analysis enables a fluorescence threshold to be determined which defines the tumor margins. Thus, the surgeon can control the resection margins intraoperatively [35].

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