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Abstract Many medical applications, including photodynamic therapy for cancer (PDT), involve the use of lasers. However, the coherence of laser light is not necessary for PDT, and attempts have been made to construct non-coherent light sources for PDT, which are relatively inexpensive, stable and easy to operate, require simple maintenance but differ fundamentally from the lasers in their output characteristics. In the present work we compared two clinically used lamps, CureLight1, which is a broadband source (560–740 nm) based on a filtered halogen lamp, and CureLight2, which is a narrowband source based on light-emitting diodes (LEDs), with respect to several parameters of crucial significance for PDT efficiency in vivo: (a) depth of action in tissues, (b) heating effects, (c) pain generation, (d) photodegradation of PpIX in solution, in cells and in mouse skin and (e) photo-inactivation of cells in vitro. We conclude that CureLight2 (LED), relative to CureLight1 (halogen) has deeper PDT action in tissue, similar efficiency for bleaching PpIX in mouse skin, better efficiency for bleaching PpIX in cells and solutions and good efficiency for inactivating cells in vitro. CureLight2 gives less heating of the tissue and less pain in unsensitised human skin. All these differences are related to difference in the spectra of the lamps. Thus, PDT light sources with emissions that are visually similar have significantly different photobiological properties.

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Abbreviations ALA: 5-Aminolevulinic acid · HSA: Human serum albumin · LED: Light-emitting diode · MAL: Methyl 5-aminolevulinate · PBS: Phosphate-buffered saline · PDT: Photodynamic therapy · PpIX: Protoporphyrin IX · SDS: Sodium dodecyl sulphate

Introduction

A combination of light and chemicals is widely used to treat skin diseases. The concept of photodynamic therapy (PDT) was introduced for the main branch of this use [1–3]. PDT is a promising modality for management of tumours and non-malignant skin diseases. It is based on the administration of a photosensitiser that selectively localises in the target tissue. Exposure of the lesion to visible light in the presence of oxygen results in photodamage and subsequent tissue destruction.

Shortly after the invention of lasers in 1960, they were brought into medical use [4]. Fundamental features, such as coherence and monochromaticity of laser light, made them excellent tools for a number of applications: in surgery [5], treatment of haemangiomas [6], skin rejuvenation [7], hair removal [8], etc. Since coherence is lost within a few tenths of a millimetre of penetration into human tissue, this property is not necessary for PDT [9]. Non-coherent light is, therefore, frequently used for irradiation of neoplasms [10]. Non-coherent light sources differ fundamentally from lasers in output characteristics. Lasers and non-coherent light sources have been used for PDT [11–14] and usually show similar efficacies [15, 16]. Non-coherent light sources are relatively inexpensive, stable and easy to operate and require little maintenance. Non-coherent, filtered, light

sources often emit light with a larger bandwidth than that of lasers and light-emitting diodes (LED). A comparison of two such light sources is, therefore, not straight forward. Careful dosimetric considerations are required.

Several light sources have been used for PDT with 5-aminolevulinic acid (ALA) or its derivatives. The aim of the present study was to compare two light sources (CureLight1 and CureLight2) with respect to their effect in vitro and in vivo. The systems were sensitised by protoporphyrin IX (PpIX), induced by one of ALA derivatives, methyl 5-aminolevulinate.

We compared the efficiencies of the lamps CureLight1 (broadband halogen) and CureLight2 (narrow band, based on, LEDs) by means of three different methods.

Calculations using the fluorescence excitation spectra (F) of the photosensitiser PpIX in living tissues (mouse skin), optical penetration spectra (P) of the tissue and the emission spectra of the lamps (L) were made. If the concept of total effective fluence is considered [14], the area under the curve $F(\lambda)\times P(\lambda, d)\times L(\lambda)$ then gives the photodynamic efficiency $E(d)$ as a function of the depth (d) under the tissue surface:

$$E(d) = \int F(\lambda) \times P(\lambda, \mathrm{d}) \times L(\lambda) \times \mathrm{d}\lambda,$$

where λ is the wavelength and the integration is carried out over the entire emission range of the lamps.

Photodegradation of a photosensitiser (photobleaching) and photo-inactivation of cells are supposed to be linearly related to the generation rate of singlet oxygen [17]. It has been proposed to use photobleaching for clinical dosimetry [18–23]. Thus, we measured the photobleaching rate of PpIX exposed to light from the two lamps. The measurements were carried out for PpIX in a protein solution, in cells in vitro and in mouse skin in vivo.

We compared the efficiency of the light sources for inactivating cells incubated with ALA derivative in vitro.

The temperature during light exposure plays a significant role in PDT efficiency. Therefore, we measured the temperature of cell suspension and human skin during exposure to the lamps. We investigated human skin instead of mouse skin for two reasons: firstly, animal anaesthesia may lead to temperature changes and, secondly, animals cannot describe sensations during light exposure (burning, pain, discomfort). Anaesthesia is needed during light exposure with laboratory animals. The action of anaesthesia depends on the type and amount of anaesthetic. Most anaesthetics used for experimental animals lower their metabolic activity [24]. We noticed that the intraperitoneally injected anaesthetic Hypnorm–Dormicum induced a reduction in skin temperature, lasting until the animals woke up and became normal and active [25]. The action of anaesthetics is different for individual animals, and it is hard to maintain the same depth of anaesthesia [26].

Materials and methods

Light sources

CureLight1 V 1.5 (serial No. 01000138, PhotoCure ASA, Oslo, Norway) is a broadband lamp for clinical use in topical PDT of skin lesions. It consists of three main parts: a filtered halogen lamp, a control panel and a power supply. The recommended exposure area is 38 mm in diameter, and the distance between the lamp and the skin surface should be 80 mm. This distance between the lamp and the sample surface or photodetector was maintained in all the measurements.

CureLight2 V 3.1 (serial No. 00100011, PhotoCure ASA) is a so-called LED lamp intended for clinical use in topical PDT of skin lesions. It consists of three main parts: a lamp unit containing the LED, a control panel and a flexible arm with a base and a power supply. The recommended exposure area is 50×65 mm, and the distance between the lamp and the skin surface should be 50 mm. This distance between the lamp and the sample surface or photodetector was maintained in all the measurements.

Fluence rate measurements

The fluence rate of the lamps was measured with two different instruments: a thermopile (Power Max 5200 Laser Power Meter, Molectron Detector, Portland, Ore., USA), which measures both visible and infrared (IR) radiation; the fraction of IR radiation was measured by means of a silicon filter (0.5 mm thickness, transmission region 1,200–2,700 nm); and the PowerMeter photodiode (NewPort, Model 1815-C, Irvine, Calif., USA), which measures only visible light.

The lamps were switched on for at least 15 min before the measurements were taken. Then, the detectors were placed in front of the light sources. The results are presented as averages ± standard error of three independent measurements performed on three different days.

Spectroscopic measurements of the emission spectra of the lamps

The emission spectra of CureLight1 and CureLight2 (Fig. 1) were measured with a fibre-optic probe connected to a luminescence spectrometer (Perkin Elmer LS50B, Norwalk, Conn., USA). The fibre-optic probe is a commercially available bifurcated fibre accessory (Perkin Elmer; two 1 m fused silica fibre bundles joined in parallel at the measuring tip) fitted with a cylinder-shaped aluminium spacer (6.5 mm diameter), which provides a constant fixed distance of 10 mm between the fibres and the sample. This assures a relatively uniform light distribution over the measuring area and provides

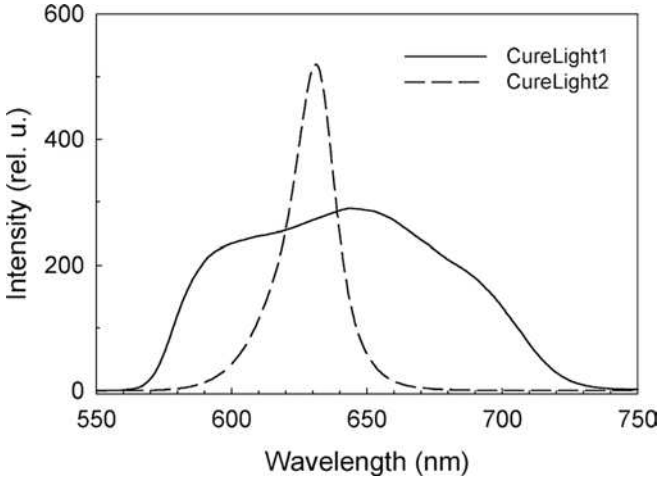

Fig. 1 Emission spectra of CureLight1 and CureLight2

the maximum fluorescence signal for the given set-up. The emission spectra are corrected for the spectral sensitivity of the spectrometer and the photomultiplier (R3896, Hamamatsu, Japan). The calibration was performed with a light source (LS-1-CAL, Ocean Optics, Dunedin, Fla., USA) with a known emission spectrum.

Chemicals

Methyl 5-aminolevulinate (MAL) and Metvix cream (containing 160 mg/g MAL) were supplied by PhotoCure ASA. RPMI-1640 medium, penicillin/streptomycin solution, L-glutamine, trypsin-EDTA, phosphate-buffered saline (PBS) and other chemicals were obtained from Sigma-Aldrich Norway AS (Oslo, Norway). Foetal bovine serum was obtained from GIBCO BRL (Life Technologies, Roskilde, Denmark). PpIX was obtained from Porphyrin Products (Logan, Utah, USA). All chemicals were of the highest purity that was commercially available. A total of 10 mM stock solution of MAL in RPMI-1640 medium was prepared ex-tempera before every experiment and then diluted to a final concentration of 1 mM.

Preparation of PpIX in solution

We prepared stock solutions of PpIX (10^{-3} M) by dissolving the dye in a solvent of 1N $HClO_4$ and CH_3OH (1:1 v/v) with 1% of CH_3-$(CH_2)_{11}$-O-SO_3^- Na^+ (sodium dodecyl sulphate, SDS). The stock solution was diluted with phosphate buffered saline (PBS) to the desired concentration. To prevent aggregation, we complexed PpIX with protein by the addition of 1 mg/ml of human serum albumin (HSA).

In order to avoid inner-filter effect we ensured that all solutions used for in vitro studies had an absorbance of less than 0.1 OD throughout the whole spectrum. Absorption spectra of the PpIX solutions were measured with a UV/VIS spectrophotometer (Lambda 15, Perkin Elmer).

Cell cultivation

Human WiDr cells derived from a primary adenocarcinoma of the rectosigmoid colon [27] were used. The cell cultivation procedure was similar to that already described [28]. The cells were maintained in exponential growth phase in RPMI 1640 medium with 10% foetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin, and 2 mM L-glutamine. The cells were incubated in cell culture flasks (Nunc A/S, Roskilde, Denmark) or 35 mm-cell culture dishes (Easy-Grip FALCON, England) at 37°C in a humidified 5% CO_2 atmosphere and subcultured twice a week with 0.01% trypsin in 0.02% EDTA.

Animals

The animal experiment was performed similarly to that already described [29]. Female Balb/c athymic hairless mice were obtained from the animal department of the Radium Hospital (Oslo, Norway). At the start of the experiment the mice were 7–8 weeks old and had an average body weight of 20–25 g. In order to facilitate proper application of the cream we anaesthetised all animals with a subcutaneous injection (0.02–0.05 ml per animal) of a mixture of Hypnorm (Janssen Pharmaceutica, Tilburg, The Netherlands) and Dormicum (Hoffmann-La Roche AG, Basle, Switzerland) (1:1 v/v). All spectroscopic measurements were performed while the mice were under anaesthesia. Metvix cream (containing MAL at concentrations of 160 mg/g) was supplied by PhotoCure in 2 g aluminium tubes. Approximately 0.1 g/cm^2 of Metvix was applied topically on the left flank of each mouse. The cream was evenly distributed and covered with a transparent dressing (Opsite Flexigrid, Smith and Nephew Medical, Hull, England). The mice were fed with a standard rodent diet and kept in a 12 h on–off light cycle, except for during the application of Metvix when they were kept in the darkness before the measurements.

One female RWT hairless rat (nu/nu, approximately 8 weeks, 100 g) obtained from the animal department of the Radium Hospital was used for measurements of light penetration in rodent skin.

Fluorescence measurements

The fluorescence of PpIX in solutions or in cells in vitro was measured directly with the luminescence spectrometer. The fluorescence of skin in vivo was measured with the fibre-optic probe connected to the luminescence spectrometer. Excitation wavelength was set at 407 nm,

corresponding to the maximum of the Soret band of the PpIX excitation spectrum, and fluorescence emission was measured at 635 nm. In addition, we measured fluorescence excitation and emission spectra to verify that the fluorescence signal originated mainly from PpIX. The 407 nm excitation light from the luminescence spectrometer was of low intensity (less than 1 mW/cm^2) and did not induce any significant photobleaching of PpIX. The excitation and emission slits were set at 5 nm and 10 nm, respectively. A 515 nm cut-off filter was used in the emission optical path of the instrument during detection of the emission spectra.

Photobleaching of PpIX in solution

The PpIX solutions were irradiated with CureLight1 and CureLight2 from PhotoCure. PpIX fluorescence was measured in cuvettes ($d = 10$ mm) placed directly in the standard cuvette holder of the luminescence spectrometer. The fluorescence background (autofluorescence) of the solvent without PpIX was recorded and subtracted from the fluorescence data.

Photobleaching of PpIX in the cells

Approximately 10^6 cells were seeded per one Nunclon flask (Nunclon, Denmark) and incubated for 2 days at 37°C for proper attachment to the substratum. After 2 days the cells were exposed to 1 mM MAL in culture medium without serum for 5 h to allow build-up of intracellular porphyrins. After incubation with MAL the cells were brought into suspension by being scraped. They were exposed to light from either CureLight1 or CureLight2.

The PpIX fluorescence in the treated cells was measured in cuvettes ($d = 10$ mm) placed directly in the standard cuvette holder of the luminescence spectrometer. The fluorescence background (autofluorescence) of untreated control cells was recorded and subtracted from the fluorescence data.

Survival of the cells

The cell survival assay was similar to that described earlier [28]. Approximately 500 cells were seeded into 35 mm cell culture dishes (Easy-Grip, FALCON) and incubated for 2 days at 37°C for proper attachment to the substratum. Subsequently, they were incubated with 1 mM MAL dissolved in the culture medium without serum for 5 h. The cells then were irradiated with CureLight1 or CureLight2.

After irradiation, the cells were incubated in fresh medium with serum for 10 days for colony formation. At the end of the incubation period, they were rinsed with 0.9% NaCl solution, fixed in absolute ethanol, stained with methylene blue and counted.

We determined the survival of the cells by counting the number of colonies formed after 10 days of incubation. In total, three experiments were performed, with three parallels in each experimental group.

Photobleaching of PpIX in mouse skin

The transparent dressing was removed from each mouse before exposure to light from CureLight1 or CureLight2. Prior to cream application the fluorescence background (autofluorescence) of skin was recorded from each animal and subtracted from the fluorescence data. Fluorescence spectra from the skin of the mice were measured with the fibre-optic probe 3 h after cream application, immediately before and after light exposure.

Temperature measurements in solutions and on human skin during light exposure

A total of 2 ml of RPMI 1640 medium was added into 35 mm cells culture dishes with cells and brought to 37°C (as for the cells in the incubator). Prior to light exposure, while a thermocouple (Kane-May KM457XP, UK) was fixed in the correct position in the solution and the dish was covered, the temperature decreased to 32–33°C. The conditions were very similar to those of the cell survival experiment. The temperature of the solution was measured continuously with the thermocouple during light exposure.

The temperature of human skin was measured with the thermocouple during light exposure on the lower arms of four healthy volunteers. Light exposure was interrupted for few seconds, and the thermocouple was pressed against the skin with a small piece of heat insulating material (isopor).

Light penetration in skin

Light penetration was measured as described earlier [30, 31]. Briefly, light from a quartz halogen lamp (10 W) was transmitted through a perspex rod into a fold of rat skin and transferred through a 600 μm quartz fibre to the luminescence spectrometer. The distance between the quartz fibre and the perspex rod, i.e. the thickness of the skin fold, was accurately controlled by a micrometric screw. The spectra of the light were recorded for different skin thicknesses.

Results

Spectroscopic characteristics of the lamps

CureLight1 is a broadband lamp (560–740 nm; Fig. 1). The average fluence rate of CureLight1 was 285 ± 6 mW/cm^2, when measured with the thermopile,

and 240 ± 2 mW/cm^2, when measured with the photodiode. The difference, 43mW/cm^2 (15%), between these measurements corresponds well to the fraction of IR radiation (38 mW/cm^2, 13%) measured when we used the silicon filter.

CureLight2 is a lamp with the spectral range 580–670 nm and peak wavelength at 631 ± 2 nm (Fig. 1). The average fluence rate of CureLight2 was 90 ± 4 mW/cm^2, when measured with the thermopile, and 90 ± 2 mW/cm^2, when measured with the photodiode. This lamp does not emit IR. (See Table 1)

Efficiency of the lamps calculated at various skin depths

For the comparison of the lamps with respect to efficiency the light penetration spectrum (Fig. 2) and the PpIX fluorescence excitation spectrum [31] were used. The efficiency of the lamps at different depths was calculated as described in ''Materials and methods''. The transmission E to different tissue depths (D) was fitted to an exponential function:

$$E = E_0 \cdot e^{-\frac{d}{\delta}}.$$

The constant δ describes the depth where the irradiance is decreased by a factor $e \approx 2.7$ compared with the irradiance E_0 incident on the skin surface (Fig. 2).

Effective excitation of PpIX

The effective excitation of PpIX at various tissue depths with the excitation beam falling perpendicularly on the skin surface was calculated by use of the penetration spectrum (Fig. 2) and the fluorescence excitation spectrum F_0 of PpIX in cells:

$$F_{\text{eff}} = F_0 \times e^{-\frac{d}{\delta}}.$$

Effective fluence of CureLight1 and CureLight2 for PpIX excitation

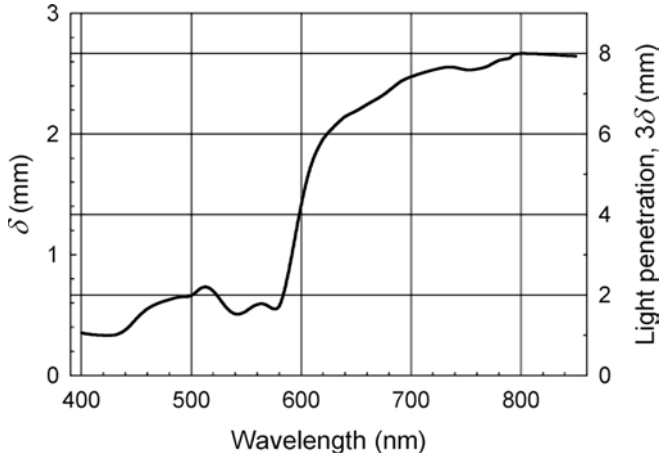

Fig. 2 Light penetration in rat skin in vivo

In order to calculate effective fluence of the lamps, we multiplied the emission spectra (L_0) of the lamps (Fig. 1) by the effective excitation spectrum of PpIX (Fig. 3):

$$L_{\text{eff}} = L_0 \times F_0 \times e^{-\frac{d}{\delta}}.$$

We determined the spectral efficiency by integrating over all wavelengths for various depths (calculating the area under the spectra with SigmaPlot software). CureLight2 is more effective than CureLight1 for depths greater than 1.5 mm, whereas the opposite is true for smaller depths (Table 2, Fig. 4).

Temperature measurements in cells suspensions and on human skin during light exposure.

CureLight1 emits approximately 13% IR of the total radiation emitted (according to the fluence rate measurements). Therefore, the temperature changes were tested in cell solution and in human skin during light exposure (Fig. 5).

During light exposure with CureLight1 the temperature of the cell solution increased by 8 ± 2°C, whereas in the case of CureLight2 the temperature decreased to the room temperature (26 ± 1°C) (Fig. 5a).

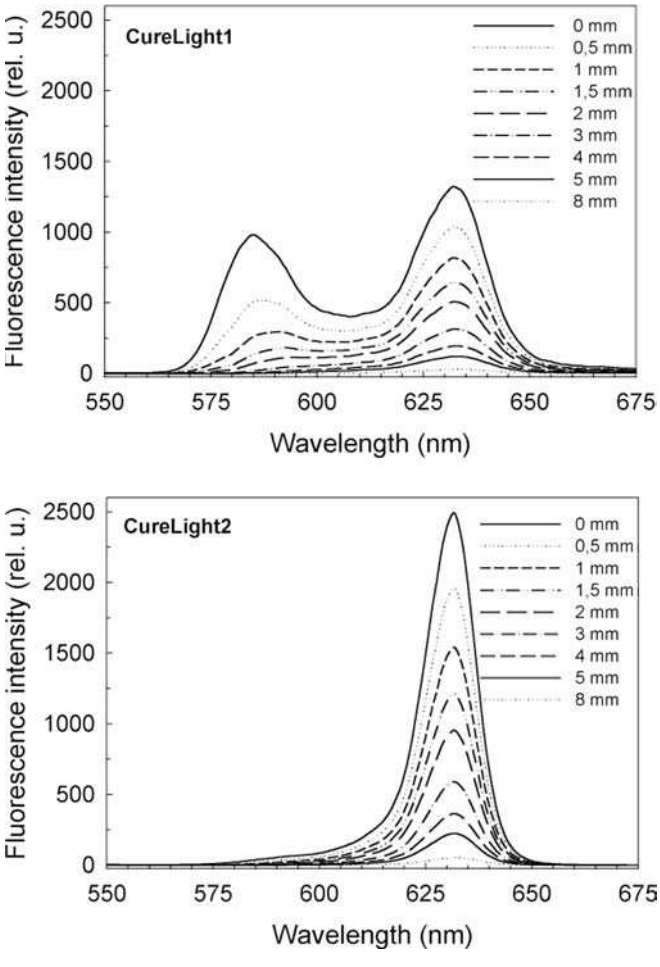

Fig. 3 Effective fluence of CureLight1 and CureLight2 for PpIX excitation at various tissue depths

Table 1 Characteristics of the lamps

Parameters	CureLight1	CureLight2
Thermopile measurement		
Fluence rate	285 ± 6 mW/cm^2	90 ± 4 mW/cm^2
Fraction of IR radiation	38 ± 1 mW/cm^2 (13%)	0 mW/cm^2
Photodiode measurement		
Fluence rate	240 ± 2 mW/cm^2	90 ± 2 mW/cm^2
Spectral range	570–740 nm	580–660 nm
Peak emission	Broadband	631 nm
Area of light exposure	38 mm diameter	50×65 mm
Recommended distance between the lamp and sample	80 mm	50 mm
Temperature increase of human skin after 10 min light exposure	9 ± 1°C	1 ± 1°C

Table 2 Theoretical calculated area under the spectra (software: SigmaPlot 7.1, SPSS, Chicago, Ill., USA) of the lamps (Fig. 3) at various tissue depths

Tissue depth (mm)	Spectral area (relative units)		CureLight2/ CureLight1
	CureLight1	CureLight2	
0	53,774	41,078	0.76
0.5	37,082	31,978	0.86
1	26,448	24,922	0.94
1.5	19,335	19,442	1.01
2	14,386	15,179	1.06
3	8,237	9,271	1.13
4	4,842	5,673	1.17
5	2,887	3,476	1.20
8	636	804	1.26

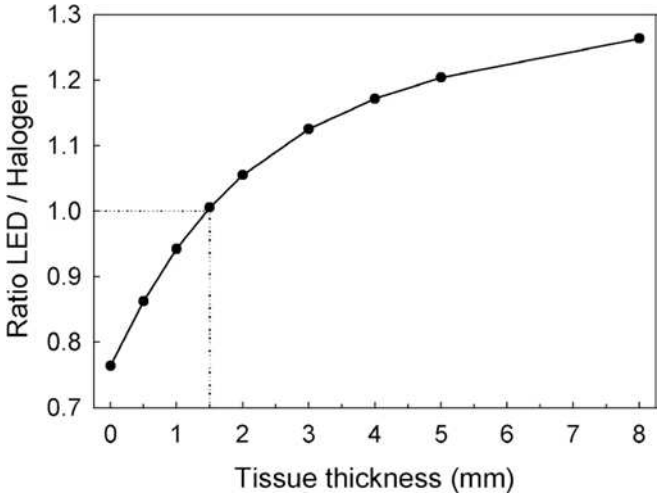

Fig. 4 Relative efficiency of CureLight1 and CureLight2 at different skin depths

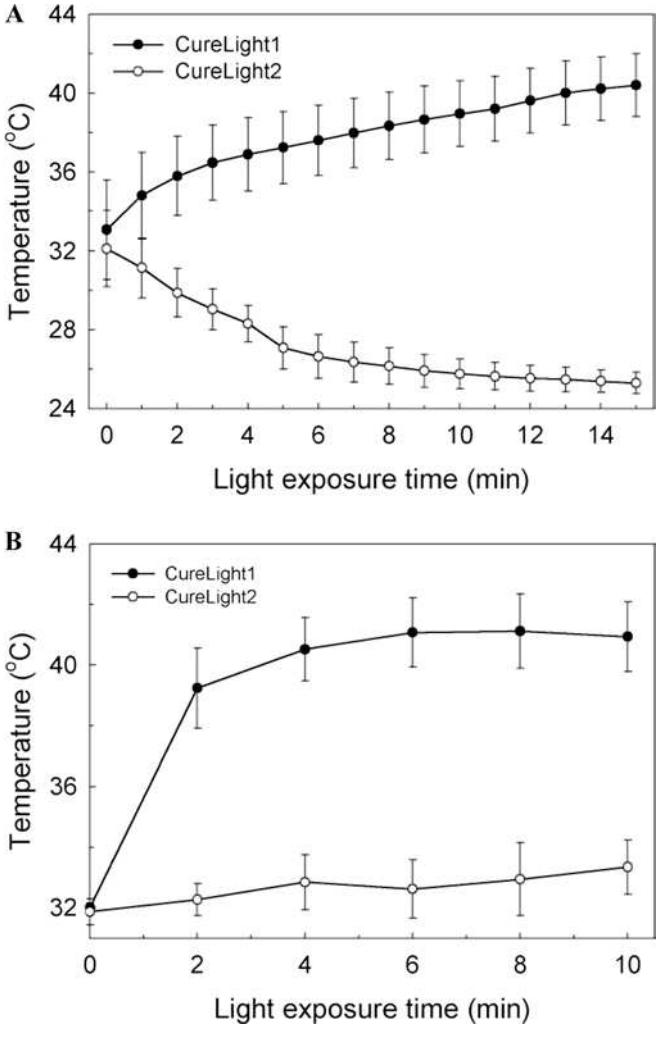

Fig. 5 Temperature measurements in the cell culture medium (**a**) and on the arm of a healthy person (**b**) during exposure to CureLight1 and CureLight2 lamp

On healthy human skin a slight redness was observed after light exposure for 5 min with CureLight1. No redness was observed under exposure to light from CureLight2. All volunteers reported slight pain and a slight burning sensation in the case of CureLight1 during first minutes, but not in the case of CureLight2. During light exposure the temperature on the healthy human skin increased by 9 ± 1°C for CureLight1 and 1 ± 1°C for CureLight2 (Fig. 5b).

Effectiveness of the lamps

CureLight2 was more effective in photobleaching PpIX and in inactivating cells than CureLight1 (Table 3). The fluence needed to kill the same number of cells in vitro was lower for CureLight2 than for CureLight1 (Fig. 6).

All controls were measured (light–no drug; drug–no light; no drug–no light), and no statistically significant differences were noted.

The fluence needed to photobleach the same amount of PpIX in solution, in cells in vitro, or in mouse skin in vivo was lower for CureLight2 than for CureLight1 (Fig. 7).

Discussion

The shapes of the survival curves provide important information about the capacity of the cells to accumulate sublethal damage and their sensitivity to light. Analysis of the dose–response curves reveals differences in the curve shoulder and in the slope for the two light sources (Fig. 6). CureLight1 gives a larger shoulder (11.3 J/cm^2) and a lower slope ($D_o = 16.3$ J/cm^2) than CureLight2 (3.4 J/cm^2 and $D_o = 4.9$ J/cm2, respectively) (Fig. 7). Thus, CureLight2 seems to be more efficient in the inactivation of cells than CureLight1.

Since both photobleaching and tumour destruction are dependent on singlet oxygen generation, the rate of photobleaching can be used to estimate the therapeutic efficiency [32]. We measured the photobleaching during light exposure in order to clarify if this state was reached and to evaluate the dosimetric usefulness of photobleaching. The largest light doses used in our study were sufficient to degrade almost all PpIX in solution, in cells and in healthy mouse skin (Fig. 7).

As can be seen from Table 4 the fluences needed to bleach 90% of PpIX in skin are different, as has been found in different investigations. This might partly be due to the fact that light sources with different fluence rates and spectral properties were used. However, the fluences given for human basal cell carcinoma (BCC) are exceptionally low. This indicates that the oxygen concentration in these tumours is high or that PpIX is in microenvironments that favour photodegradation. The bleaching rates may also be related to the fluence rate. A high fluence rate may lead to rapid oxygen depletion, resulting in slow bleaching, or to heating effects, which also may change the bleaching rates. However, except for our CureLight1 experiment, the fluence rates used in these studies were below the hyperthermia limit (150 mW/cm^2) [33, 34]. The fluence given by Robinson et al. [35] for mouse skin exposed to light at 514 nm (25 J/cm^2) is about a factor of 3.2 smaller than that found by us for exposure at approximately 630 nm (CureLight2). This difference is related to the extinction

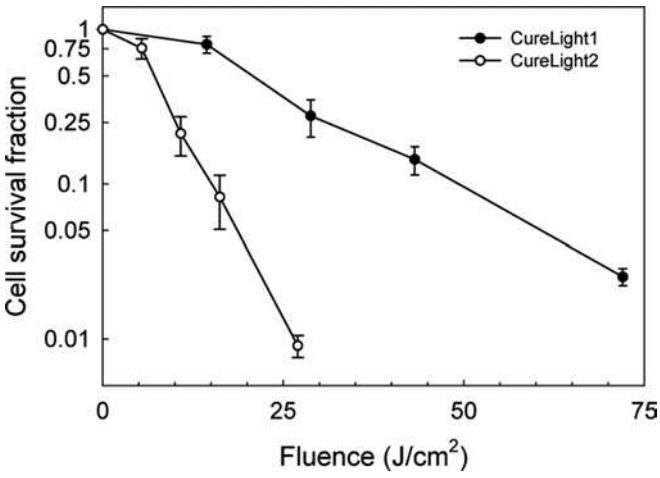

Fig. 6 Survival of WiDr cells exposed to PDT. Light–dose response. WiDr cells were exposed for 5 h to 1 mM MAL in a serum-free medium. Photoradiation was performed at room temperature (22°C). Data represent mean colony counts of three culture dishes ± standard errors (SE) derived from three individual experiments

coefficient of PpIX at the two wavelengths. The non-aggregated fraction of porphyrins in solution is likely to be a fluorescent and photoactive one [36–38]. Thus, fluorescence excitation spectra should be used to estimate relative extinction coefficient at different wavelengths. For PpIX in cells the fluorescence excitation value is a factor of 2.7 larger at 514 than at 630 nm. This leads to a better agreement between our work and that of Robinson et al. [35].

The damage caused by photodynamic therapy cannot be predicted by the total light exposure [20, 41, 42], due to oxygen depletion at high fluence rates [43]. More tissue damage and faster bleaching is observed at low fluence rates [20, 41, 42].

Photosensitising photoproducts are formed from PpIX in organic solvents, in cell cultures and in tissues [44–49]. We did not observe any photoproducts, possibly because the spectra of the lamps cover the absorption spectra of the photoproducts, which are also photolabile [50].

There is no good correlation between photoin activation of cells and photobleaching of the PpIX in them. CureLight2 was, relatively to CureLight1, more efficient in the bleaching of PpIX in the cells than in the inactivation of the cells. This might partly be related to the need of higher light doses for bleaching than for inactivation (Table 3). Inactivation may be related to a subfraction of

Table 3 Ratio of fluences of CureLight1 and CureLight2 needed to bleach an equal fraction of PpIX in different systems or to kill the same fraction of cells

The fractions are given in the second line

Experiment	Ratio fluences (CureLight1/CureLight2)				Average (±SD)
	25%	50%	75%	90%	
PpIX bleaching in solution	1.59	1.88	2.23	2.24	2.0 ± 0.3
PpIX bleaching in WiDr cells	2.33	2.04	2.28	2.27	2.2 ± 0.1
PpIX bleaching in mouse skin	1.00	1.00	1.17	1.30	1.1 ± 0.1
Cell survival	2.87	2.90	3.09	3.26	3.0 ± 0.2

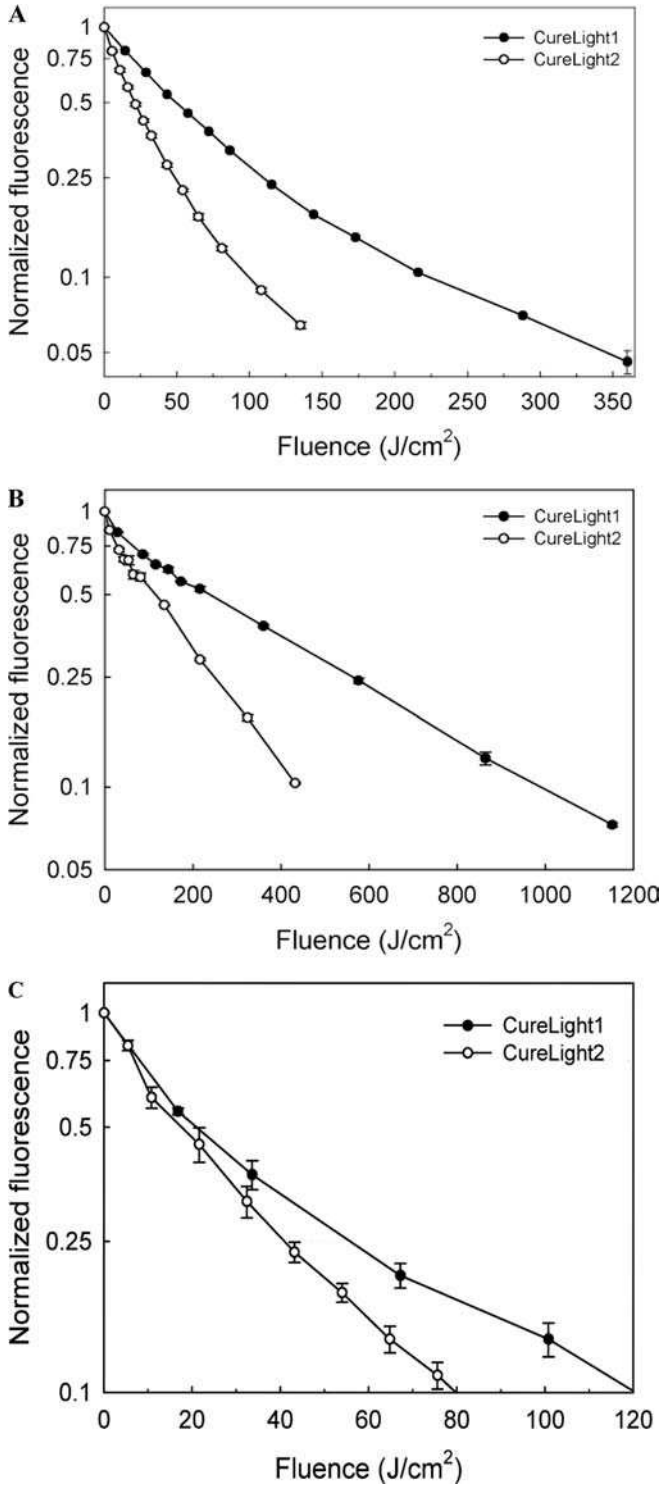

Fig. 7 Photobleaching kinetics of PpIX in solution (**a**), in WiDr cells (**b**) and in mouse skin (**c**)

PpIX in the cells. It is known that sensitisers are relocated in cells during PDT [51–53]. Differences in binding sites lead to differences in photolability [54].

CureLight2 is, relatively to CureLight1, more efficient in the bleaching of PpIX in cells than of mouse skin. This might be related to light absorption in the tissue,

Table 4 Photobleaching of PpIX using different light sources

Experiment	Light source	Spectrum	Drug (topical)	Light fluence rate	Fluence needed to bleach 90% of PpIX	Reference
In humans						
Superficial BCC lesions	Broadband lamp	570–740 nm	20% ALA for 3 h	165–175 mW/cm^2	20 J/cm^2	[15]
Superficial BCC lesions	Copper vapour laser pumping a dye laser	630 nm	20% ALA for 3 h	155 mW/cm^2	15 J/cm^2	[15]
In mice						
Normal skin of hairless SKH-1 mice	Slide projector (Kodak 650 H)	VIS	8% MAL for 1.5 h	10 mW/cm^2	30 J/cm^2	[39]
Normal skin of hairless SKH-1 mice	Slide projector (Kodak 650 H)	VIS	8% MAL for 1.5 h	40 mW/cm^2	45 J/cm^2	[39]
UV-induced tumours in hairless SKH-1 mice	Slide projector (Kodak 650 H)	VIS	8% MAL for 1.5 h	10 mW/cm^2	30 J/cm^2	[39]
UV-induced tumours in hairless SKH-1 mice	Slide projector (Kodak 650 H)	VIS	8% MAL for 1.5 h	40 mW/cm^2	41 J/cm^2	[39]
Normal skin of albino hairless SKH1 HR mice	Argon ion laser	514 nm	20% ALA for 4 h	50 mW/cm^2	25 J/cm^2	[35]
Normal skin of albino hairless SKH1 HR mice	Argon ion laser	514 nm	20% ALA for 6 h	50 mW/cm^2	25 J/cm^2	[35]
Normal skin of Balb/c mice	CureLight1 (LED)	580–660 nm	Metvix for 5 h	90 mW/cm^2	80 J/cm^2	Our study
Normal skin of Balb/c mice	CureLight2 (broadband)	570–740 nm	Metvix for 5 h	250 mW/cm^2	120 J/cm^2	Our study
Normal skin of Balb/c mice	Dye laser	635 nm	20% ALA	100 mW/cm^2	70 J/cm^2	[40]

i.e. to the local space irradiance. Fluorescence is mainly detected from the superficial layers of the skin (excitation at 407 nm) where CureLight1 gives, relatively to CureLight2, higher space irradiance.

ALA PDT is generally well tolerated by patients and gives good cosmetic results, although some patients feel pain during light exposure [55–57]. Fijan et al. [58] and, Clark et al. [16] found that the pain increased with increasing fluence, whereas Grapengiesser et al. [57] reported that the pain did not depend on the light dose. The reason for this discrepancy is not known. A possible reason might be the skin temperature increase during PDT. In our study all volunteers reported slight pain and a slight burning sensation in the case of CureLight1 (broadband) but not in the case of CureLight2 (LED), even when no PpIX was present in the tissues. The skin temperature increased from 32 to 41°C during 3–4 min exposure to CureLight1 at 240 mW/cm^2 (Fig. 6b). This should not give hyperthermia effects [59]. The temperature increase in the irradiated area was a result of direct absorption of the light by tissue chromophores (melanin, haemoglobin, etc.) in the skin. Svaasand [35] showed that there were no hyperthermic effects when fluence rates below 150 mW/cm^2 at a wavelength of 514 nm were used. Warloe et al. [60] measured the skin surface temperature during PDT, using fluence rates of 100–150 mW/cm^2 at a wavelength of 630 nm, and showed that there were no hyperthermic effects under their experimental conditions.

Since the penetration depth of light decreases for short wavelengths (see Fig. 2), the exposed tissue volume will be considerably smaller for wavelengths shorter than 580 nm than for red light (> 620 nm). As can be seen from Table 2 and Fig. 4, CureLight2 acts deeper in the tissue than CureLight1. Thus, CureLight1 will act in a small volume and give high local fluence rates. This may stimulate nerves in the superficial layers. Red light (the spectrum of CureLight2) is dissipated in a larger volume, and local fluence rates are likely to be below the thresholds for photothermal injury and pain.

Pain during PDT is a consequence of nerve stimulation, possibly by reactive oxygen species (ROS). ROS and $^1O_2$ will be formed in a larger volume during exposure to red light than to green light. Since pain is a rather individual feeling, it is difficult to assess during PDT. Treatment of actinic keratoses by green light was less painful than treatment with red light [61]. However, Morton et al. [62] found no significant difference in pain when they used red and green light to treat patients with Bowen's disease.

Since inactivation of the lower part of a tumour, i.e. its base, is almost always the main problem in PDT, CureLight2, with its larger effective penetration depth (Figs. 3 and 4), is likely to be the better lamp. It may also be advantageous, since it causes less pain than CureLight1. Unwanted IR present in a broadband non-coherent light source might lead to unwanted heating of tissues [63, 64]. Furthermore, the presence of IR might introduce dosimetric problems, since many detectors also register IR. On the other hand, the presence of IR might be favourable, since PDT and sublethal hyperthermia have been shown to have a synergistic effect [65, 66].

In conclusion, in our experimental models CureLight2 has more favourable characteristics for PDT with ALA or its derivatives than CureLight1.

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