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Short communication

Potency of different red light sources in photodynamic induction of cell death in a squamous cell carcinoma cell line

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ABSTRACT

LED illumination systems were found to be more efficacious than broad spectrum lamps in recent phase III trials on photodynamic treatment of actinic keratoses. However, a detailed comparison of the light doses emitted at the appropriate spectral range and its correlation to photodynamic effects is thus far not available for the most frequently used devices. Here, we compared the spectral emissions of three different PDT lamps with their potency of inducing cell death in ALA-loaded A431 cells, including a new system equipped with more advanced LEDs matching the photosensitizer absorption peak more precisely and emitting more homogeneous light over time. Cells were exposed to two different ALA concentrations, incubated for 1 or 3 h and then illuminated by one of two different LED or a broad-spectrum system at four different light doses, whereupon viability was assessed. Maximal doses were selected in accordance to clinically applied light doses in recent phase III studies and the manufacturers’ recommendations. The data gathered here clearly demonstrate that the two LED systems were significantly more effective in inducing cell death than the broad spectrum system. Most efficient was the newer LED system, in agreement with emission parameters that more accurately corresponded to the photosensitizer’s absorption peak.

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1. Background

The efficacy of PDT of skin neoplasia depends on PpIX concentration, oxygen, and light dose (J/cm²). The PpIX absorption peak around 635 nm seems ideal for dermatologic applications since red light penetrates deeper into skin than light hitting absorption peaks at shorter wavelengths [6].

Several red light systems are routinely used for PDT in Europe. Most frequent are narrow spectrum LED lamps emitting around 630/635 nm and a broad spectrum lamp emitting a continuous spectrum above 590. Recent clinical comparisons of the clinical efficacies achieved with these lamps proved the higher efficacy of LED lamps [2,3,8].

2. Aims

The intention of this study was to analyze and compare commercially available light sources for PDT. Light intensities and spectral distributions were measured and correlated to the respective efficacies in killing ALA-loaded A431 cells. The results shall provide insight into the correlation between the light dose at 635 nm and in vitro and clinical efficacy.

3. Methods

Lamp types and light doses were applied as follows:
A Gigahertz BTS 256-LED device was used to measure spectral distribution in W/nm. Test distances were chosen according to the respective user manuals: 6 cm for the LED systems and 30 cm for the broad spectrum lamp.

To record light intensity curves in Lux over 10 min, a Gossen Mavalux 5032C USB luxmeter was used at the distances indicated above.
A431 cells were cultivated and exposed to 5-aminolaevulinic acid (ALA) as described previously [7] at the intervals and concentrations indicated below (Table 2). Times and concentrations were selected according to previous experiments [7].

Experiments were independently repeated on 5 days with every data point performed in triplicates.
Following incubation, A431 cells were subjected to different light doses (Table 1). Immediately afterwards, cell viability was
Table 1
Light fluence rates.

<table>
<thead>
<tr>
<th>Lamp</th>
<th>Aktilite CL128</th>
<th>BF-RhodoLED</th>
<th>PhotoDyn 750</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light fluence in J/cm² (time)</td>
<td>3 (46 s)</td>
<td>3 (49 s)</td>
<td>7.5 (38 s)</td>
</tr>
<tr>
<td>6 (1 min, 32 s)</td>
<td>6 (1 min, 37 s)</td>
<td>15 (1 min, 15 s)</td>
<td></td>
</tr>
<tr>
<td>15 (3 min, 51 s)</td>
<td>15 (4 min, 3 s)</td>
<td>75 (6 min, 15 s)</td>
<td></td>
</tr>
<tr>
<td>37 (9 min, 30 s)</td>
<td>37 (10 min)</td>
<td>170 (14 min, 10 s)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Incubation periods and concentrations.

<table>
<thead>
<tr>
<th>Time</th>
<th>Concentration</th>
<th>Aktilite CL128</th>
<th>BF-RhodoLED</th>
<th>PhotoDyn 750</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h without 5-ALA (vehicle)</td>
<td>3 h without 5-ALA (vehicle)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h at 0.6 mM 5-ALA</td>
<td>3 h at 0.6 mM 5-ALA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h at 6.0 mM 5-ALA</td>
<td>3 h at 6.0 mM 5-ALA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Measurement of physical lamp parameters. (a) Spectral emission of the three analyzed lamps measured in W/nm. Light intensities were assessed and plotted against wavelength in the range of visible light from 380 to 740 nm. (b) Light intensity emitted from the three analyzed lamps over the time course of an illumination leading to an emitted light dose of 37 J/cm² (LED lamps) or 170 J/cm² (broad spectrum lamp). Intensities were measured in lux and plotted against time in seconds.

Results were analyzed using the Mann-Whitney U test. Each lamp was compared to each other lamp in a dose-matched pair wise fashion. Significance criteria were met at p-values < 0.05.

5. Conclusion

Phase III studies revealed that PDT with both ALA and MAL is more effective with LED devices than broad band devices [2,3,8], and this study was performed to explain this difference achieved with standard PDT lamps. At about 635 nm the LED lamps generated a light intensity of 9 mW/cm², the broad-spectrum device PhotoDyn only 0.3 mW/cm², which may be the cause for lower potency in the induction of cell death in vitro and in phase III trials [1,3,4,8]. A similar difference had been postulated for LED and IPL devices [5]. While both LED lamps had a comparable impact on cell viability, BF-RhodoLED displayed a tendency towards higher efficacy in this test than Aktilite and achieved a significant difference to the broad-spectrum system at the lower light dose (Fig. 2c). The PhotoDyn device was far less efficacious in this test similar to the results of phase III trials with these devices. As the maximal light intensities (37 J/cm² and 170 J/cm²) matched clinically relevant light doses [8], these findings may explain the outcomes of published phase III studies.

In conclusion, the abovementioned difference in clinical efficacy is most likely related to lower light intensity at the relevant wavelength, where the broad band lamp displays about 30-fold lower light intensity than the LED lamps. Since this light intensity was insufficient to induce cell death here, it is surprising that clinical efficacy is still achieved. In two published phase III trials on PDT treatment of actinic keratosis using a 10% ALA in a nanoemulsion formulation (BF-200 ALA), the proportion of completely cleared patients was 84.8% and 87% with LED (mostly Aktilite) and 71.5% and 53% with broad band devices [1,3,8]. Unpublished clinical data on the efficacy of BF-RhodoLED in field treatment of actinic keratoses resulted in 91% of completely cleared patients.

Conflict of interest

BN is an employee of Biofrontera Pharma GmbH, the company which commercializes the BF-RhodoLED light source. MP is an employee and HL is the CEO of Biofrontera AG, the company which developed the BF-RhodoLED light source. TB is an employee of Scemtech Sensir Technology, a company that was contracted by Biofrontera during the development process of the BF-RhodoLED light source.
Fig. 2. Induction of cell death. Cell viability was normalized to control conditions in [%] plotted against light dose in j/cm². The first value in the x-axis labels describes the dose applied from LED lamps, the second one describes that of the broad spectrum lamp. (a) Cell viability after illumination at different light doses after 1 h incubation with 0.6 mM ALA. (b) Cell viability after illumination with the different light doses after 1 h incubation with 6 mM ALA. (c) Cell viability after illumination with the different light doses after 3 h incubation with 0.6 mM ALA. (d) Cell viability after illumination with the different light doses after 3 h incubation with 6 mM ALA. Viability is expressed as mean value from five independent experiments with three data points each ± S.E.M. For pairwise comparison of Aktilite vs Photodyn: * = p < 0.05; ** = p < 0.01. For pairwise comparison of BF-RhodoLED vs Photodyn: +++ = p < 0.001.

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References