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A Possible New Incoherent Lamp for Photodynamic Treatment of Superficial Skin Lesions

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Since coherence of laser light is not necessary for photodynamic therapy of skin lesions, attempts have been made to construct incoherent lamps. A recent development is the PDT 1200 (Waldmann Medizintechnik/Germany), a light source consisting of a 1200 watt metal halogen lamp. Emission of 600 to 800 nm radiation is achieved by using cut-off filters. Power density can be varied from 30 mW/cm² to 200 mW/cm² in an area from 100 to 300 cm². Biological effectiveness was proved by comparison with the radiation of an argon-pumped dye laser (Kiton red) emitting light at 630 nm. Three human cell lines were incubated with photofrin at different concentrations. After irradiation, cell viability was tested (MTT assay). Results proved biological effectiveness of the light source PDT 1200. No significant difference in cell viability was detected using either concentration of sensitizer. Therefore, we believe that PDT 1200 is a promising new light source for photodynamic therapy of skin lesions. ***Key words: MTT assay; Dye laser; Photofrin; Hematoporphyrin derivative.***

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In recent years, photodynamic therapy (PDT) has shown effectiveness in the curative and palliative treatment of cancer (1). PDT works by way of photosensitized molecules of light at certain wavelengths (e.g. at 630 nm) causing energy-dependent cytotoxicity. Based on tumour location (skin surface, hollow organs, etc.) different light application systems for PDT are necessary. A variety of systems are currently available, using either laser sources for irradiation (argon-pumped dye lasers) or incoherent wavelength-filtered lamps. Since coherence of light is not mandatory for skin surface illumination, and irradiation with lamps is more reliable and simpler and cheaper than with lasers, the only problem that remains to be solved is how to increase the intensity level achievable (currently only about 50 mW/cm²) to that of lasers (150–200 mW/cm²) (2). The aim of the present study was to prove the biological effectiveness of the new PDT 1200 lamp for PDT.

MATERIAL AND METHODS

Human cell lines (HaCaT (3), J82 (4), foreskin fibroblasts (HF)) were maintained at 37°C in a humidified atmosphere (5% CO_2) in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin (400 U/ml), streptomycin (50 μg/ml), glutamine (400 μg/ml), and ascorbate (50 μg/ml). Cells were grown in 96 cell cultures (7×10^4 cells per dish). Cells were allowed to attach overnight, then the medium was removed. One hundred μl of serum-free medium, containing photofrin (Photofrin Medical Inc., Raritan, NJ, USA) at a concentration of either 5 μg/ml or 10 μg/ml was added to the cultures, and the cells were allowed to take up the dye for 24 h at 37°C. Extracellular dye was removed and the cells were rinsed once with PBS and then covered with PBS. Light treatment was performed immediately afterwards. Irradiation was performed with either a dye laser or the PDT 1200 lamp (Waldmann Medizintechnik, VS-Schwenningen, Germany), emitting incoherent light. This light source is a 1200 watt metal halogen lamp (MSR 1200, Philips BV, Eindhoven, NL) – emission of 600 to 800 nm radiation is achieved by using dichroic cut-off filters (DT Rot and Calflex-3000, Balzers Optik, Nürnberg, Germany) (Fig. 1). The wavelength band was limited because most of the photosensitizers of clinical interest have strong absorption bands at 630 nm (2). Shorter wavelengths were not used, because they do not reach deeper layers in the skin. Infrared radiation was excluded, because of heating effects during irradiation. Depending on distance between light source and skin surface, intensity could be varied from 30 mW/cm² to 200 mW/cm² in an irradiated area from 100 to 300 cm². Intensity measurements (laser powermeter, Ophir Optics Ltd., Jerusalem, Israel) showed a homogeneous gaussian distribution with an intensity loss of less than 10% in the central light spot (95 cm², distance 100 cm). An argon-pumped dye laser (2040 and 375, Spectra Physics Lasers, Inc., Mountain View, CA, USA), exciting Kiton red dye and producing up to 3.5 W of red light at $\lambda = 630$ nm, was used for comparison. The fluence rate to which cells were exposed was adjusted in both light sources to 40 mW/cm². Depending on exposure time and according to preliminary dose-dependent studies, a light dose of 30 J/cm² was selected. After photoirradiation, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was immediately performed to check cell viability (described in sections (5, 6)). After 24 h, optical densities of the wells were read using an ELISA reader at 540 nm as a parameter of cell viability. Nine groups were formed for each cell line ($n = 24$ for each group). Group 1 served as a control and received no sensitizer or irradiation. Group 2 received irradiation with dye laser only, group 3 with PDT 1200 only. Group 4 was treated with 5 μg/ml photofrin, group 5 with 10 μg/ml photofrin. Group 6 was treated with 5 μg/ml photofrin and dye laser, group 7 with 5 μg/ml photofrin and PDT 1200. Group 8 was treated with 10 μg/ml photofrin and dye laser, group 9 with 10 μg/ml photofrin and PDT 1200. The StatView II data analysis system (Abacus Concepts, Inc., Berkeley, CA, USA) was used for statistical analysis of the data. Descriptive statistics, normality tests, one-factor analysis of variance parameters, and the Scheffé F-test were used.

RESULTS

Optical densities of all groups were compared to those of the untreated group (100%). Irradiation of cell lines only with PDT 1200 or dye laser showed no significant difference in cell viability to controls (group 2: HaCaT: $80 \pm 3.7\%$ (S.E.M.); HF: $96 \pm 2.5\%$; J82: $116 \pm 5.5\%$) (group 3: HaCaT: $84 \pm 6.9\%$; HF: $100 \pm 2.5\%$; J82: $108 \pm 5.2\%$). Incubation with photofrin at both concentrations without irradiation (dark toxicity) led to a significantly ($p < 0.05$) lower cell viability than controls in J82 cells, but not in HaCaT cells, and in HF cells only at 10 μg/ml photofrin concentration (group 4: HaCaT: $127 \pm 8.0\%$; HF: $111 \pm 8.6\%$; J82: $36 \pm 1.9\%$) (group 5: HaCaT: $84 \pm 9.0\%$; HF: $28 \pm 6.1\%$; J82: $24 \pm 1.9\%$). Light irradiation of photofrin-incubated cells led to a significant decrease in cell viability at different concentrations compared to non-illuminated cells and

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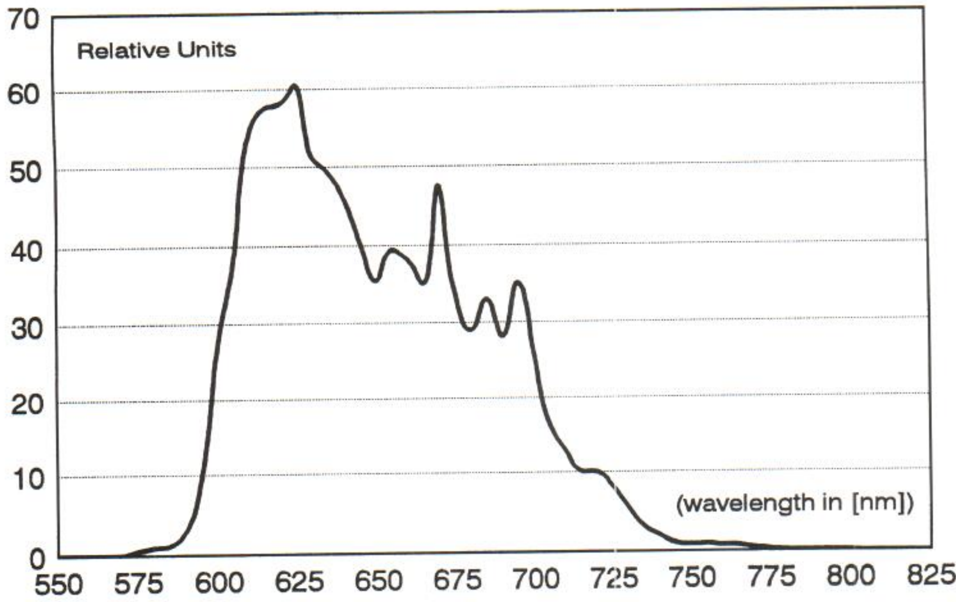

Fig. 1. Relative spectral power distribution of PDT 1200 lamp.

controls ($p<0.001$). No significant difference in cell viability was detected between laser and PDT 1200 irradiation of photofrin-incubated cells at either concentration (group 6: HaCaT: 1.3±0.2%; HF: 1.6±0.2%; J82: 1.3±0.2%) (group 7: HaCaT: 2.8±0.5%; HF: 3.4±1, 1%; J82: 2.9±0.5%) (group 8: HaCaT: 1.9±0.3%; HF: 3.8±1.7%; J82: 0.8±0.2%) (group 9: HaCaT: 8.0±2.3%; HF: 8.9±5.1%; J82: 3.0±1.7%) (Fig. 2).

DISCUSSION

PDT is of increasing interest, and in several in vitro and in vivo studies it has been demonstrated that incoherent light sources are effective (7–9). Up to now, however, it has only been possible to irradiate small areas with the incoherent light sources available. In contrast, the newly constructed light source PDT 1200 is comparable to the argon-pumped dye laser with respect to physical parameters such as power density and irradiatable area.

As expected, no significant change in cell viability was detected in cells irradiated only with laser or PDT 1200. A significant decrease in cell viability was seen in J82 cells incubated with photofrin at both concentrations without irradiation (dark toxicity). This effect is possibly due to the long incubation period and high concentrations of sensitizer, resulting in inhibition of microtubule assembly (10). However, fibroblasts and HaCaT cells at 5 µg/ml photofrin incubation showed an increased cell viability above 100%. Although this effect is not of statistical significance, photofrin seems to induce cell growth under certain conditions.

Current research shows that the mechanism of phototoxicity by photofrin is a result of production of highly reactive intermediate singlet molecular oxygen, leading mainly to membrane damage of sensitized and irradiated cells (11). After longer incubation periods, photofrin is mainly localized to the mitochondria, where, after light activation, it harms membranes and reduces the activity of membrane-associated enzymes like cytochrome C oxidase and succinic dehydrogenase (8, 12). Lack of

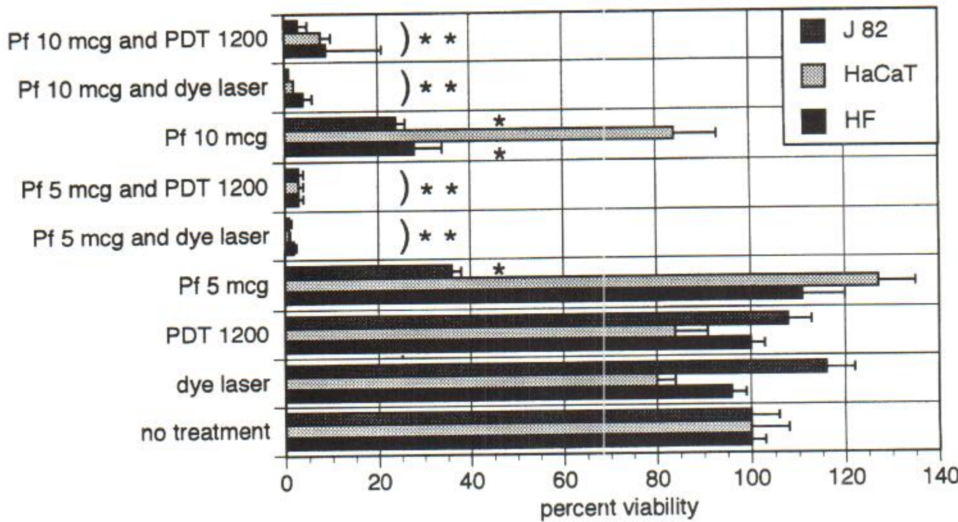

Fig. 2. Cell viability of three different cell lines after incubation with 5 µg/ml and 10 µg/ml photofrin for 24 h. Controls served as 100%. Comparison between irradiation (light dose 30 J/cm²) with dye laser and incoherent light source (PDT 1200) shows no significant difference. Significant dark toxicity for photofrin incubation alone (* $p<0.05$, ** $p<0.001$ error bars: S.E.M.).

enzymatic activity of succinic dehydrogenase after PDT in vitro could easily be determined by an MTT assay (8).

The incoherent lamp revealed a comparable biological effectiveness in the three cell lines, since cell viability after laser or PDT 1200 irradiation did not differ significantly. We believe, therefore, that PDT 1200 is a promising new light source for the treatment of superficial skin lesions with PDT.

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