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Verapamil enhances the uptake and the photocytotoxic effect of PII, but not that of tetra(4-sulfonatophenyl)porphine

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Abstract

The influence of the calcium channel blocker verapamil on the sensitivity of mouse fibrosarcoma cells of the line EMT-6 to treatment with Photofrin II (PII) or tetra(4-sulfonatophenyl)porphine ($TPPS_4$) and light has been assessed. Cells were treated with 1.5 μg/ml PII or 75 μg/ml $TPPS_4$ overnight in the absence or presence of 50 μg/ml verapamil and subsequently exposed to light. Verapamil increased the sensitivity of the EMT-6 cells to PII-induced photoinactivation by a factor of 2. In contrast, verapamil decreased the sensitivity of the cells to $TPPS_4$-induced photoinactivation by 50–60%. Both sensitizers were found to be located to a large extent in lysosomes as revealed by fluorescence microscopy and by photochemical inactivation of the lysosomal marker enzyme β-*N*-acetyl-D-glucosaminidase. Verapamil increased the uptake of PII by 30% and reduced the uptake of $TPPS_4$ by 20%. Furthermore, verapamil enhanced the binding and uptake of LDL by about 40%. In conclusion, the effects of verapamil-induced sensitization of EMT-6 cells treated with PII or $TPPS_4$ and light can to a large extent be attributed to the modulatory effects of verapamil on endocytosis. © 1998 Elsevier Science B.V.

Keywords: Photodynamic therapy; Photofrin II; Tetraphenylporphine; Verapamil; Lysosome

1. Introduction

Photodynamic therapy (PDT) is an experimental treatment modality which presently is undergoing clinical trials [1,2]. The cytotoxic effects are suggested to be mainly mediated through the formation of singlet oxygen, oxidizing unsaturated fatty acids, cholesterol, some amino acids as well as guanine [3]. The subcellular damage introduced by singlet oxygen will depend on the intracellular localization of the photosensitizer.

Calcium homeostasis has been found to be perturbed in cells after photochemical treatment with several photosensitizers [4–7]. The increased intracellular free Ca^{2+} has been suggested to act as a cytoprotective response in T24 cells through formation of prostaglandins and c-AMP, which can act as membrane stabilizing agents [8]. On the other hand, in L929 cells attenuation of the increased free calcium induced by PDT reduced the sensitivity of these cells to PDT [9]. Another approach to study the importance of calcium in the cytotoxic effects of

Abbreviations: β-AGA, β-*N*-acetyl-D-glucosaminidase; HpD, hematoporphyrin derivative; NR, neutral red; PII, Photofrin II; $TPPS_4$, tetra(4-sulfonatophenyl)porphine; $TPPS_{2a}$, *meso*-tetraphenylporphine with 2 sulfonate groups on adjacent phenyl rings

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PDT is to combine PDT with calcium channel blockers, like verapamil and diltiazem [10]. Biade et al. [10] found that these drugs potentiated the sensitivity of HT29-18 cells to PII-induced photoinactivation. In this study it was concluded that the sensitization induced by the calcium channel blockers was not due to enhanced uptake of PII which could have occurred due to the effect of verapamil on the 170 kD MDR glycoprotein or on the uptake of LDL [11–14]. To further elucidate the mechanisms of verapamil-enhanced sensitization of cells treated with PDT another cell line has been selected and the effect on PII-PDT has been compared with PDT using $TPPS_4$ as photosensitizer.

2. Materials and methods

2.1. Chemicals

Photofrin II (PII) was provided by PII Medical (Raritan, NJ), and $TPPS_4$ by Porphyrin Products (Logan, UT). Verapamil and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide were purchased from Sigma (St. Louis, MO). All chemicals were of the highest purity available.

2.2. Cell cultivation

Mouse fibrosarcoma cells of the line EMT-6 [15] were cultivated in HAM F-10 medium containing 10% foetal calf serum and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin). The cells displayed a doubling time of 10–12 h and were subcultured at least twice a week.

2.3. Labelling with photosensitizers, verapamil and irradiation

The cells were seeded out and incubated overnight for proper attachment to the substratum. The next day the cells were incubated with 75 μg/ml $TPPS_4$ or 1.5 μg/ml PII in the presence or absence of 50 μM verapamil (if not otherwise described) for 18–20 h. The cells were washed 3 times with 0.9% NaCl before exposure to light. Cells treated with PII were exposed to UVA light at a fluence rate of 2 mW/cm^2 with a Vilber–Lourmat TF-35L system while cells treated with $TPPS_4$ were exposed to light using an illumination table (30 × 25 cm) thermostated at 37°C and having two 500 W tungsten–halogen lamps whose light was filtered through Balzers R 65 optical filters (600 nm long-pass filter). The fluence rate was 7 mW/cm^2. Fluence rates for both lamps were determined by chemical actinometry using the photosensitized degradation of histidine as actinometer [16]. Treatment with light alone at the doses given here does not cause any reduction in cell survival.

2.4. Cellular uptake of PII and $TPPS_4$

80×10^4 cells were seeded out in 20 cm^2 dishes and incubated overnight for proper attachment to the substratum. The next day the cells were incubated with the sensitizer as described above. At the end of the incubation period the cells were washed 3 times with PBS. Cells treated with $TPPS_4$ were scraped off the substratum in 1 ml of PBS and 100 μl 1 M NaOH were added and mixed into the solution. $TPPS_4$ was measured spectrofluorometrically (Perkin-Elmer LS-5) using a 1 ml cuvette and the fluorescence was measured by exciting the samples at 420 nm and detecting the emission at 648 nm. Cut-off filters were used both on the excitation (345 nm) and on the emission (545 nm) side to avoid contributions from second order light. Cells treated with PII were scraped off the substratum in 1 ml of 1% Triton X-100. PII was measured as $TPPS_4$ except that PII was excited at 403 nm and fluorescence detected at 629 nm. For quantification a standard of known concentration was added to the samples to increase the fluorescence by 50–100%. The cellular uptake studies were based on established procedures [17,18] and the results analysed by Student's *t*-test.

2.5. Cytotoxicity assay

Approximately 2000 cells/cm^2 were seeded out in 10 cm^2 dishes (Nunclon) and treated as described above. Cytotoxicity was measured by the neutral red assay as previously described [19,20]. A stock solution of 0.4% of neutral red was diluted 40 times in HAM F-10 medium (without serum) the day before performing the assay. The diluted neutral red (NR)

solution was centrifuged before use to remove neutral red crystals. Immediately after photodynamic treatment the cells were washed twice with saline (0.9% NaCl) and the cells were incubated with 500 μl of the NR solution for 2 h. Subsequently, the cells were washed twice with saline and incubated for 2 min in 500 μl 0.4% formaldehyde containing 10% $CaCl_2$. The formaldehyde solution was then sucked off and 1 ml of a mixture containing 50% ethanol, 20% acetic acid and 30% H_2O was added. NR uptake was measured spectrophotometrically at 540 nm using a Perkin-Elmer Lamda 5 spectrophotometer.

2.6. Fluorescence microscopy

Ten-cm^2 dishes (Falcon 3001) were used in the microscopical studies. The cells were washed once with PBS and a cover glass was gently put on top of a PBS layer. The cells were subsequently studied by a Zeiss Axioplan microscope equipped with epifluorescence. A HBO/100 W mercury lamp was used for excitation. The cells and the cellular fluorescence were studied by the means of a cooled charge-coupled device (CCD) camera (TE2, Astromed, Cambridge). A computer controlled the camera operation and was used for digital image processing and storage. The microscope was equipped with a 390–440 nm band pass excitation filter, a 470 nm dichroic beam splitter and a 610 nm long pass filter.

2.7. β-N-acetyl-D-glucosaminidase (β-AGA) assay

Cells used for enzymatic measurements were trypsinized, pelleted and seeded out on 10 cm^2 dishes (Falcon 1008) (1×10^6 cells in HAM F-10 medium (containing 10% serum)). The cells were then irradiated and pelleted immediately after irradiation. β-AGA activity was measured as described by Beaufay et al. [21]. The method is based on the formation of *p*-nitrophenol (from the substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) which can be registered spectrophotometrically at 410 nm. The amount of photosensitizer in the cells did not influence on the measurements as also found in a previous study [22].

2.8. LDL preparation, labeling and binding assay

LDL, taken as the 1.024–1.050 fraction, was prepared from sera of healthy volunteers by sequential ultracentrifugation according to Havel et al. [23]. LDL labeling was performed with [^{125}I]Na by the method of Bilheimer et al. [24]. The specific radioactivity was about 300 dpm/ng. LDL binding (at 4°C) or binding and internalization (at 37°C) were assayed according to Goldstein and Brown [25]. To this purpose, the cells were precultured for 24 h in Ham F10 medium supplemented with 2% Ultroser G and verapamil when indicated, then washed 3 times with PBS, and incubated either for 1 h at 4°C (binding) or for 4 h at 37°C (binding plus internalization) in the presence of ^{125}I-LDL as indicated on the figures or figure legends, in 0.5 ml Ham F10 medium, pH 7.4, supplemented with 2% Ultroser G and 25 mM Hepes buffer, in the absence or in the presence of a large excess (0.25 mg/ml) of unlabeled LDL. Experiments were stopped on ice. Cells were washed 3 times with cold PBS, then dissolved in 0.1 N NaOH. The cell-associated radioactivity was measured using a 1275 minigamma LKB gamma counter. The specific binding or binding plus internalization was calculated as follows: (cpm/mg cell protein measured in the absence of unlabeled LDL) − (cpm/mg cell protein measured in the presence of unlabeled LDL). The results are expressed as ng ^{125}I-LDL/mg cell protein. Protein determination was done by the technique of Peterson [26].

3. Results

In a previous study verapamil was found to enhance the PII-induced photosensitization of HT29-18 cells as measured by a chromate release assay [10]. Since the influence of verapamil on the cellular metabolism and in particular the pharmacology of PII might be cell line dependent another cell line, the mouse mammary tumor line EMT-6, was selected in this study. In accordance with the results from the HT29-18 cells 50 μM, but not 10 μM verapamil enhanced the photosensitivity of PII-treated EMT-6 cells as measured by another cytotoxicity assay, i.e., the neutral red uptake assay (Fig. 1a). Verapamil was usually given overnight together with the photosensitizer. Cells treated with verapamil (50 μM) only 1 h before light exposure was equally sensitive to PDT as cells treated with PII and light only. Verapamil was

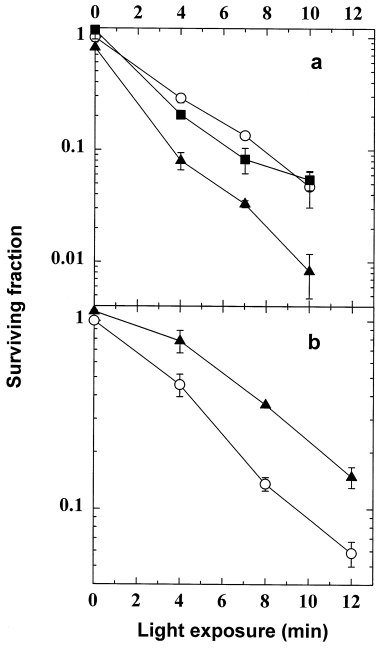

Fig. 1. Viability of EMT-6 cells exposed to PII (a) or $TPPS_4$ (b) in the absence (○) or presence of 10 μM (■) or 50 μM (▲) verapamil. Bars, SE from a representative experiment performed in duplicate.

usually removed from the medium immediately before exposure to light. However, exposure of cells to light in the presence of a medium containing verapamil did not further sensitize the cells to photoinactivation. The cellular uptake of NR was not influenced by cotreatment with 50 μM verapamil (data not shown).

PII is a mixture of porphyrin derivatives of which dihematoporphyrin ether or esters are suggested to be the most efficient in sensitizing cells to photoinactivation [27,28]. To further study the influence of verapamil on PDT another photosensitizer, $TPPS_4$, with different physico-chemical properties as well as intracellular localization from PII, was selected. Surprisingly, verapamil reduced the sensitivity of the cells to $TPPS_4$-induced photoinactivation (Fig. 1b).

Verapamil has previously been shown to influence not only the calcium homeostasis but also the uptake and metabolism of LDL [11,13]. A large fraction of PII is bound to LDL and cellular uptake is suggested to be influenced by the presence of LDL, while $TPPS_4$ is practically not bound to LDL [29,30]. The sensitizing properties of verapamil could therefore be due to effects on cellular uptake of the photosensitiz-

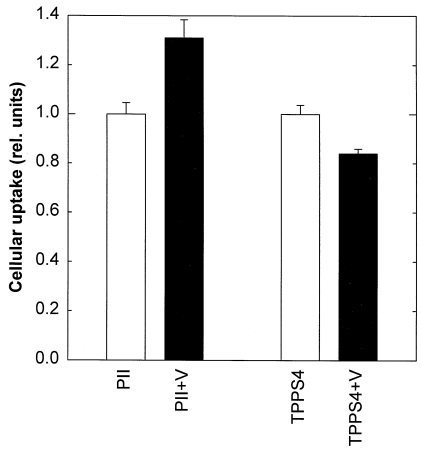

Fig. 2. Cellular uptake of PII or $TPPS_4$ in the absence or presence of 50 μM verapamil (V) as indicated on the figure. The results are normalized to that of cells treated in the absence of verapamil. Bars, SE from 4 independent experiments performed in duplicate.

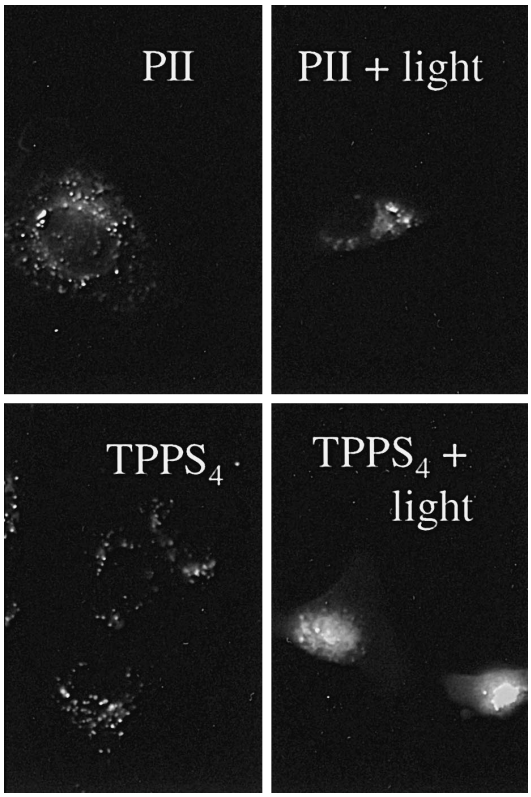

Fig. 3. Fluorescence micrographs of EMT-6 cells treated with PII or $TPPS_4$ as indicated on the figure. Cells exposed to light were evaluated 30 min after light exposure. The light doses reduced the cellular viability by approximately 50%.

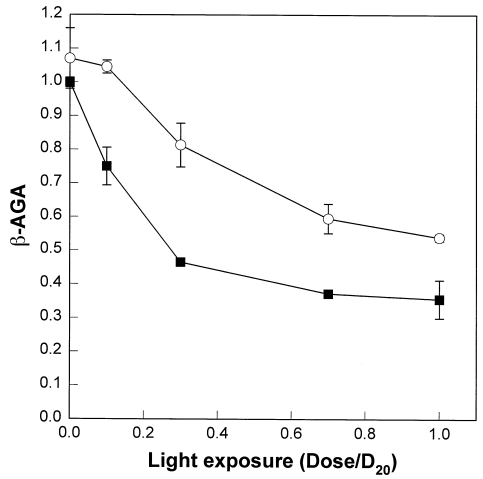

Fig. 4. Relative β-AGA activity in EMT-6 cells treated with PII (○) or $TPPS_4$ (■) and light normalized to the dose inactivating 80% of the cells (D_{20}). The β-AGA activity was normalized against the activity in cells treated with $TPPS_4$ alone. Exposure of cells to light alone has no effect on β-AGA activity.

ers. Cellular uptake studies showed that this was indeed the case (Fig. 2). Verapamil was found to enhance the uptake of PII by 30% ($p < 0.05$), while the uptake of $TPPS_4$ was reduced by approximately 20% ($0.05 < p < 0.1$).

$TPPS_4$ is in most cell lines due to endocytic uptake located in lysosomes [31]. Fluorescence microscopic studies (Fig. 3) in combination with studies of photochemical inactivation of the lysosomal marker enzyme β-AGA (Fig. 4) confirms that $TPPS_4$ is lysosomally located in the EMT-6 cells. The granular fluorescence pattern from the cellbound $TPPS_4$ resembles also that of lysosomes [32]. Furthermore, the short lifetime and diffusion length of singlet oxygen, formed by $TPPS_4$ and light, in a cellular environment strongly indicate that inactivation of β-AGA is most likely due to lysosomally located $TPPS_4$ [33]. On the other hand, PII is usually diffusely located extranuclearly in cells [34] or only located partly in lysosomes [35]. However, in EMT-6 cells PII was located granularly, resembling the localization of $TPPS_4$. Photochemical inactivation studies of β-AGA confirmed that PII was to a large extent located in lysosomes. The apparently lower effect of photochemically activated PII, than that of $TPPS_4$, on the inactivation of β-AGA may be due to the more hydrophobic properties of PII [22]. PII may associate to a higher extent with the lysosomal membranes than $TPPS_4$ and thus may locate more distantly from β-AGA than $TPPS_4$ as was found for $TPPS_{2a}$ [36]. Additionally, some lysosomes, named telolysosomes, may not be involved in fusion with endosomes and β-AGA in these vesicle cannot be photochemically inactivated [37,38]. The intracellular localization of PII and $TPPS_4$ was not found to be influenced by the presence of verapamil (data not shown).

The verapamil-induced increase in PII uptake might reflect an increased uptake of LDL due to, e.g., an increased number of LDL receptors. Thus, the bind-

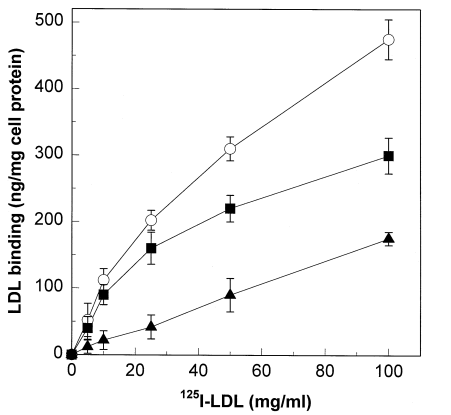

Fig. 5. Binding of [^{125}I]-labeled LDL to EMT-6 cells at 4°C as described in Section 2. Symbols: ○, total binding; ■, specific binding; ▲, non specific binding. Bars, SD from 3 experiments in duplicate.

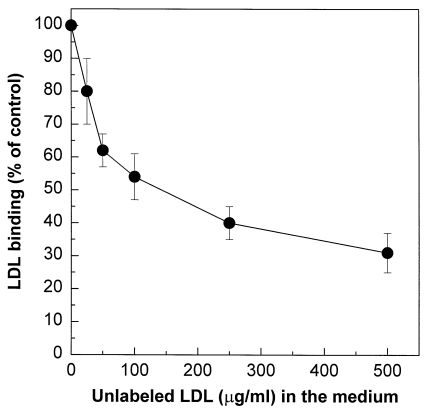

Fig. 6. Repression of the LDL receptor by exogenous LDL. The cells were cultured for 24 h in medium supplemented with various concentrations (up to 500 μg/ml) of unlabeled LDL, then washed, and the specific LDL binding was measured as specified in Section 2. Results are expressed as percentage of controls (cells cultured in the absence of LDL). Bars, SD from 3 experiments in duplicate.

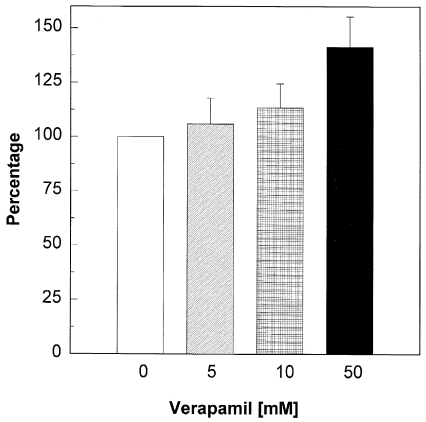

Fig. 7. Effect of verapamil on LDL binding and uptake at 37°C by EMT-6 cells. The cells were pretreated for 24 h with the indicated concentrations of verapamil. Binding and internalization of 10 μg/ml [^{125}I]-labeled LDL to EMT-6 cells at 37°C for 4 h was measured as described in Section 2. The results are presented as relative to controls. Bars, SD from 3 experiments in duplicate.

ing of the photosensitizers to the cells at 0°C was initially investigated. However, the fluorescence measurements were perturbed by high unspecific binding of the photosensitizers to the substratum introducing unreliable results. The uptake and binding of LDL in the EMT-6 cells were therefore characterized. The binding of LDL to the surface of the EMT-6 cells was found to be due to both receptor-specific binding as well as a relatively high non-specific component (Fig. 5). The expression of the LDL receptor was also found to be feedback regulated by the extracellular concentration of LDL (Fig. 6). In cells treated with verapamil there was a progressive increase in LDL binding and uptake with increasing concentrations of verapamil. After overnight treatment with 50 μM verapamil the binding and uptake of LDL was increased by 40% (Fig. 7).

4. Discussion

The calcium antagonists are usually divided into three subclasses (Class I-III), based on pharmacological activities. Verapamil, a papaverine from class I, has been shown to act on slow calcium channels. Additionally, verapamil seems to bind to the 170 kDa multidrug resistance related glycoprotein [39,14], independent of its effect on calcium ion fluxes [40]. A third property of verapamil is its modulatory effects on LDL metabolism. In several cell lines verapamil has been found to increase the binding, i.e., increase the number of LDL receptors, and internalization of LDL [11,41,13]. Degradation of LDL is often increased at low concentrations of verapamil while higher concentrations (>50 μM) may inhibit degradation through a verapamil-induced increase in lysosomal pH [11,42]. In the present study verapamil was found to increase the sensitivity of the EMT-6 cells to PII-induced photoinactivation (Fig. 1). This enhanced sensitivity may be attributed to an increased uptake of PII (Fig. 2). PII binds to a large extent to LDL and the uptake has been suggested to be related to the uptake of LDL [43,44,30]. The enhanced binding and uptake of LDL induced by verapamil in the EMT-6 cells (Fig. 7) indicate thus that verapamil stimulates LDL-mediated uptake of PII. However, the uptake of PII was increased by only 30% while the sensitivity of the cells to photoinactivation was increased by a factor of 2 (Figs. 1 and 2). Other unknown factors than total uptake may therefore also be involved in the verapamil induced sensitization.

In a previous study verapamil was found to increase the sensitivity of HT29-18 cells to treatment with Photofrin and light [10]. In this cell line the uptake of PII was not affected by verapamil and verapamil only slightly increased the uptake of LDL. The verapamil-induced stimulation of the LDL pathway seems therefore to be less effective in HT29-18 than in several other cells such as smooth muscle cells, human skin fibroblasts and hepatomas [11,13]. In HT29-18 cells verapamil may instead influence on the calcium homeostasis which has previously been shown to have impact on the sensitivity of the cells to photoinactivation [9,8]. Purkiss et al. [45] suggested that HpD was a substrate for the MDR glycoprotein, as revealed from studies of the effect of verapamil on uptake and efflux of HpD by multicellular spheroids from colorectal cell lines. In contrast, Cowled and Forbes [46] found no enhanced sensitization by verapamil of Lewis lung carcinoma cells treated with HpD and light. In these experiments verapamil was applied only 1 h before PDT together with a 10-fold higher concentration of HpD. Altogether, these results indicate that both the cell line and the experimental conditions may influence on the effect of verapamil in combination with PDT [47].

$TPPS_4$ is a hydrophilic dye with low or no affinity

for lipoproteins [29]. This is in accordance with the lack of verapamil-induced sensitization of cells treated with $TPPS_4$ and light (Fig. 1). Surprisingly, verapamil was found to desensitize cells treated with $TPPS_4$ and light (Fig. 1), most likely due to a reduced uptake of $TPPS_4$ (Fig. 2) although the sensitivity towards $TPPS_4$-PDT is reduced somewhat more than the uptake (Figs. 1 and 2). $TPPS_4$ is taken up into cells by endocytosis and locates intracellularly in endosomes and lysosomes [32,48]. There are no indications in the literature for a LDL-mediated uptake of $TPPS_4$, and $TPPS_4$ is most likely taken up by adsorptive endocytosis and pinocytosis [49], i.e., a clathrin-independent uptake. The present results may therefore indicate that verapamil attenuate the clathrin-independent pathway and stimulate clathrin-mediated endocytosis.

PII is usually found by fluorescence microscopy to be located diffusely in the extranuclear cytoplasm [34,50,51], most likely unspecifically in membrane structures [52]. Porphyrins prebound to LDL seems to be taken up to a higher extent by endocytosis and localize to some extent in endosomes and lysosomes [53,54]. In the EMT-6 cells lysosomal localization of PII was much clearer than seen in other cell lines as revealed by the subcellular localization of fluorescing PII (Fig. 3) and by the photochemical inactivation of the lysosomal marker enzyme β-AGA (Fig. 4). The short range of action of $^1O_2$, the main cytotoxic product formed in PDT, indicates that inactivation of β-AGA is due to photoactivation of lysosomally located PII [33]. There are some evidence in the literature for cell type dependent uptake and localization of photosensitizers [55,56]. The EMT-6 cells are of fibrosarcoma origin and the uptake and intracellular routing of PII in EMT-6 cells may deviate from that in other cell types. However, although intracellular localization of PII in other fibrosarcomas has so far not been described fibrosarcomas does not seem to be different from other cell types with respect to rate of uptake of PII in vitro as well as in vivo [17,57].

In summary, the present results indicate that 50 μM verapamil can potentiate the sensitivity of cells to PII-induced photocytotoxicity. In contrast, verapamil was found to attenuate the sensitivity of cells to $TPPS_4$-induced photocytotoxicity. These effects of verapamil were attributed to its modulatory effect on LDL uptake in EMT-6 cells.

Acknowledgements

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References

[1] B. Henderson, T.J. Dougherty, Photochem. Photobiol. 55 (1992) 145–157.
[2] J. Moan, K. Berg, Photochem. Photobiol. 55 (1992) 931–948.
[3] G. Jori, J.D. Spikes, in: K.C. Smith (Ed.), Top. Photomedicine, Plenum, New York, 1984, pp. 183–318.
[4] E. Ben-Hur, T.M. Dubbelman, J. Van Steveninck, Photochem. Photobiol. 54 (1991) 163–166.
[5] M. Dellinger, F. Ricchelli, G. Moreno, C. Salet, Photochem. Photobiol. 60 (1994) 368–372.
[6] P.G. Joshi, K. Joshi, S. Mishra, N.B. Joshi, Photochem. Photobiol. 60 (1994) 244–248.
[7] L.C. Penning, M.H. Rasch, E. Ben-Hur, T.M. Dubbelman, A.C. Havelaar, J. Van der Zee, J. Van Steveninck, Biochim. Biophys. Acta 1107 (1992) 255–260.
[8] L.C. Penning, J. Van Steveninck, T.M. Dubbelman, Biochem. Biophys. Res. Commun. 194 (1993) 1084–1089.
[9] L.C. Penning, J.W. Lagerberg, J.H. Van Dierendonck, C.J. Cornelisse, T.M. Dubbelman, J. Van Steveninck, Cancer Res. 54 (1994) 5561–5567.
[10] S. Biade, J.C. Maziere, R. Santus, L. Mora, C. Maziere, M. Auclair, P. Morliere, L. Dubertret, Cell. Pharmacol. 1 (1993).
[11] A. Corsini, A. Granata, R. Fumagalli, R. Paoletti, Pharmacol. Res. Commun. 18 (1986) 1–16.
[12] D.L. Frazier, M.A. Barnhill, X. Lu, E. Jones, G. Niemeyer, L. Mishu, C.D. Lothrop Jr., Lasers Surg. Med. 13 (1993) 511–516.
[13] O. Stein, E. Leitersdorf, Y. Stein, Arteriosclerosis 5 (1985) 35–44.
[14] M.C. Willingham, M.M. Cornwell, C.O. Cardarelli, M.M. Gottesman, I. Pastan, Cancer Res. 46 (1986) 5941–5946.
[15] J.D. Chapman, K. Baer, J. Lee, Cancer Res. 43 (1983) 1523–1528.
[16] M. Bazin, R. Santus, Photochem. Photobiol. 43 (1986) 235–242.
[17] W.S. Chan, R. Svensen, D. Phillips, I.R. Hart, Br. J. Cancer 53 (1986) 255–263.
[18] J.F. Evensen, J. Moan, J.W. Winkelman, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 51 (1987) 477–491.
[19] E. Borenfreund, J.A. Puerner, J. Tissue Culture Methods 9 (1985) 7–9.

[20] R.M. Lasarow, R.R. Isseroff, E.C. Gomez, J. Invest. Dermatol. 98 (1992) 725–729.
[21] H. Beaufay, A. Amar-Costesec, E. Feytmans, D. Thines-Sempoux, M. Wibo, M. Robbi, J. Berthet, J. Cell Biol. 61 (1974) 188–200.
[22] K. Berg, J. Moan, Int. J. Cancer 59 (1994) 814–822.
[23] R.J. Havel, H.A. Eder, J.H. Bragdon, J. Clin. Invest. 34 (1955) 1345–1353.
[24] D.W. Bilheimer, S. Eisenberg, R.I. Levy, Biochim. Biophys. Acta 260 (1972) 212–221.
[25] J.L. Goldstein, M.S. Brown, J. Biol. Chem. 249 (1974) 5153–5162.
[26] G.L. Peterson, Anal. Biochem. 83 (1977) 346–356.
[27] D. Kessel, P. Thompson, B. Musselman, C.K. Chang, Cancer Res. 47 (1987) 4642–4645.
[28] A.F. Mironov, A.N. Nizhnik, A.Y. Nockel, J. Photochem. Photobiol. 4 (1990) 297–306.
[29] M. Kongshaug, J. Moan, S.B. Brown, Br. J. Cancer 59 (1989) 184–188.
[30] J.C. Maziere, P. Morliere, R. Santus, J. Photochem. Photobiol. B 8 (1991) 351–360.
[31] K. Berg, in: H. Honigsmann, G. Jori, A.R. Young (Eds.), The Fundamental bases of Phototherapy, OEMF, Milano, (1996) 181–207.
[32] K. Berg, A. Western, J.C. Bommer, J. Moan, Photochem. Photobiol. 52 (1990) 481–487.
[33] J. Moan, K. Berg, Photochem. Photobiol. 53 (1991) 549–553.
[34] J. Moan, J.V. Johannessen, T. Christensen, T. Espevik, J.B. McGhie, Am. J. Pathol. 109 (1982) 184–192.
[35] M. Geze, P. Morliere, J.C. Maziere, K.M. Smith, R. Santus, J. Photochem. Photobiol. B 20 (1993) 23–35.
[36] K. Berg, J. Moan, Cancer Lett. 88 (1995) 227–236.
[37] K. Berg, K. Prydz, J. Moan, Biochim. Biophys. Acta 1158 (1993) 300–306.
[38] E. Holtzman, in: E. Holtzman (Ed.), Lysosomes, Plenum, New York, 1991, pp. 93–160.
[39] T. Tsuruo, H. Iida, S. Tsukagoshi, Y. Sakurai, Cancer Res. 42 (1982) 4730–4733.
[40] D. Kessel, C. Wilberding, Cancer Lett. 25 (1984) 97–101.
[41] R. Paoletti, F. Bernini, R. Fumagalli, M. Allorio, A. Corsini, Ann. New York Acad. Sci. 522 (1988) 390–398.
[42] S. Ranganathan, R.L. Jackson, Biochem. Pharmacol. 33 (1984) 2377–2382.
[43] C. Candide, P. Morliere, J.C. Maziere, S. Goldstein, R. Santus, L. Dubertret, J.P. Reyftmann, J. Polonovski, FEBS Lett. 207 (1986) 133–138.
[44] D. Kessel, Cancer Lett. 33 (1986) 183–188.
[45] S.F. Purkiss, M.F. Grahn, M. Turkish, M.G. Macey, N.S. Williams, Br. J. Surg. 79 (1992) 120–125.
[46] P.A. Cowled, I.J. Forbes, Br. J. Cancer 59 (1989) 904–909.
[47] L. Gossner, H. Wittke, A. Warzecha, R. Sroka, H. Ernst, M. Meier, C. Ell, Br. J. Cancer 64 (1991) 84–86.
[48] J.M. Wessels, W. Strauss, H.K. Seidlitz, A. Ruck, H. Schneckenburger, J. Photochem. Photobiol. B 12 (1992) 275–284.
[49] K. Berg, J. Moan, Photochem. Photobiol. 65 (1997) 403–409.
[50] H. Schneckenburger, A. Ruck, B. Bartos, R. Steiner, J. Photochem. Photobiol. B 2 (1988) 355–363.
[51] K.W. Woodburn, N.J. Vardaxis, J.S. Hill, A.H. Kaye, D.R. Phillips, Photochem. Photobiol. 54 (1991) 725–732.
[52] J.R. Shulok, M.H. Wade, C.W. Lin, Photochem. Photobiol. 51 (1990) 451–457.
[53] A.C. Croce, E. Wyroba, G. Bottiroli, J. Photochem. Photobiol. B 6 (1990) 405–417.
[54] P. Morliere, E. Kohen, J.P. Reyftmann, R. Santus, C. Kohen, J.C. Maziere, S. Goldstein, W.F. Mangel, L. Dubertret, Photochem. Photobiol. 46 (1987) 183–191.
[55] J.D. Austen, S. McConnell, C.J. Carrano, M. Tsutsul, Cancer Treat Rep. 62 (1978) 511–518.
[56] M. Korbelik, G. Krosl, H. Adomat, K.A. Skov, Photochem. Photobiol. 58 (1993) 670–675.
[57] M. Korbelik, G. Krosl, Br. J. Cancer 73 (1996) 506–513.