Subcellular co-localization of aluminum (III) phthalocyanine chloride tetrasulphonate with fluorescent markers in the human melanoma cell-line HT-144

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Abstract

Objective: The exposure of a photosensitizer to visible light, preferably laser light in the red region, in the presence of molecular oxygen results in a photochemical reaction described as photodynamic therapy (PDT). Photodynamic therapy is increasingly used for the treatment of skin cancers. The subcellular localization of the photosensitizer has been shown to be a key factor in the outcome of PDT. Mitochondrial localized photosensitizers are able to induce apoptosis very rapidly. Lysosomal localized photosensitizers can elicit either a necrotic or an apoptotic response. The present work contributes to the classification of the subcellular targets and cell death pathway of PDT using hydrophilic aluminum (III) phthalocyanine chloride tetrasulfonate ($AlPcS_4$).

Material and methods: The localization of $AlPcS_4$ in metastatic human melanoma (HT-144) cells was studied by means of fluorescent probes (Rh-123, LysoTracker® and Fluo-3) and a laser scanning microscope (LSM).

Results: The laser sources of the LSM induced a PDT effect with a redistribution of $AlPcS_4$ inside the entire cell, followed by the increased fluorescence of $AlPcS_4$ in the cytoplasm of HT-144 cells.

Conclusion: The results suggest that $AlPcS_4$ is a potential sensitizer for melanoma skin cancer treatments. However, a comparison of its cytotoxicity, uptake and accumulation in normal and cancer cells deserve further investigation.

Keywords: Photosensitizer; Photodynamic therapy; Phthalocyanines; Co-localization; Laser scanning microscope

Introduction

The development of ideal photosensitizers is an ongoing area of research in photodynamic therapy (PDT). An ideal photosensitizer should be a pure substance of known composition that is stable at room temperature, has a selective accumulation within tumor tissue and shows subcellular localization within tumor cell organelles. It should be not toxic to tissue or cells in the absence of light, i.e. only cytotoxic in the presence of light of defined wavelength, and absorb light of wavelengths between 650 and 850 nm for a maximum light penetration through tissue with minimal light scattering. Moreover, it should have long triplet state lifetime, high single oxygen quantum yield for photochemical event, usually production of singlet oxygen and other reactive oxygen species [1–4].

The efficacy of PDT is dependent on the uptake of a photosensitizing dye by the tumor or other abnormal tissue that is to be treated, the subsequent irradiation of the tumor with visible light of specific wavelength absorption of the dye, and presence of molecular oxygen to generate reactive oxygen intermediates [5]. Subcellular targets of photosensitizers are mitochondria, lysosomes, endoplasmic reticulum, Golgi apparatus and plasma membranes [6].

Several categories of photosensitizers have been used in PDT studies for different types of cancers with different clinical patterns in oncology [3]. Photofrin® was first approved in Canada for the treatment for esophageal cancer in 1995 [7]. It has now been approved in more than 40 countries for the treatment of advanced and early lung cancer, superficial gastric cancer, esophageal cancer, adenocarcinoma, cervical cancer, and bladder cancer. Methyl-tetrahydroxyphenyl chlorin (Temoporfin) is approved in the European Union, Norway and Iceland for palliative treatment of head and neck cancer [8]. Other approved photosensitizers are 5-aminolevulinic acid (approved in USA for actinic keratosis), Foscan® (approved in Europe for head and neck cancer), mono-L-aspartyl chlorin e6 (NPe6) (approved in Japan for early lung cancer), hypericin (in preclinical trials for bladder cancer) while silicon phthalocyanine is in phase-I clinical trial [9].

Phthalocyanines are a group of second generation photosensitizers which are structurally related to porphyrins [10]. They have been extensively studied as PDT agents because of their

favorable photophysical and photochemical properties. Phthalocyanines exhibit strong absorption bands at 670–770 nm in the red region of the electromagnetic spectrum and the presence of an appropriate central atom, such as zinc, aluminum or silicon, yields high singlet oxygen production with long-lived triplet states [3,7,9,10]. *In vitro* results, using a single well-defined cell model (human epidermoid carcinoma cell line) and comparable illumination regimes, demonstrated great differences in basic PDT-related characteristics between several, widely used photosensitizers including aluminum (III) phthalocyanine chloride tetrasulphonate ($AlPcS_4$), which was shown to absorb light at a wavelength of 674 nm providing a higher penetration depth in tissue [11].

It was shown that the type of cell death triggered by PDT is dependent on the localization of the photosensitizer in cells [12,13]. Several pathways involved in cell death as well as survival following PDT have been reviewed. Cell death by apoptosis after PDT has been demonstrated *in vitro* [14]. The apoptotic cascade, initiated in the mitochondria by the release of cytochrome C, is well characterized for several photosensitizers [15].

Laser scanning confocal microscopy has been reviewed as crucial tool for a wide range of investigations in the biological and medical sciences for imaging thin optical sections in living and fixed specimens. Membrane-impermeant fluorophores and probes for evaluating membrane integrity of cells were applied together with nuclear functions, cytoskeletal structure and organelle detection [16].

In a previous study [17], derivatives of sulfonated aluminum phthalocyanine was shown to be localized in the lysosomes of human melanoma cell line (LOX) using the laser scanning microscope (LSM). In this study, we investigated localization of hydrophilic $AlPcS_4$ in lysosomes and mitochondria of human melanoma (HT-144) cells and calcium level regulations to evaluate its potency for PDT treatment of skin melanoma-associated cancer and possible cell death mechanisms.

Material and methods

Chemicals

Hydrophilic $AlPcS_4$ (molecular weight: 895.19 g/mol) was obtained from Frontier Scientific (Logan, UT, USA). Stock solutions of 10 mM sensitizer were made up in Dulbecco's phosphate buffered saline (DPBS; Gibco, Darmstadt, Germany) and sterilized by filtration using a 0.2 µm filter. Molecular probe Rhodamine 123 (Rh-123) was from Fluka and LysoTracker® green and Fluo-3 were purchased from Invitrogen (Darmstadt, Germany). 2-

amino-3-methyl-7-dimethylaminophenazoniumchloride (neutral red, NR) was purchased from Biochrom AG (Berlin, Germany) and used according to the manufacturer's instructions.

Cell culture

Cultures of human metastatic melanoma cell line (HT-144 cells, ATCC No HTB-63^{TM}, LGC Standards GmbH, Wesel, Germany) were grown in the supplemented culture medium (Dulbecco's modified Eagle's medium, (DMEM)) with phenol red supplemented with 10 % heat-inactivated fetal calf serum (FCS) and 1 % GlutaMAX^{TM} (Gibco, Darmstadt, Germany) at 37 °C and 5 % CO_2.

Cytotoxicity test of $AlPcS_4$

The cytotoxicity of $AlPcS_4$ was determined by incubating the cells with various concentrations of the photosensitizer (5, 10, 20, 50 and 100 µM) for 24 h. Cells were seeded into 24-well tissue culture plates at a density of 4.8 x 10^5 cells/well in 1 ml of supplemented DMEM with phenol red. Cells were allowed to attach for 48 h before being washed twice with 2 ml DPBS, then photosensitized by the addition of culture medium containing 5, 10, 20, 50 and 100 µM $AlPcS_4$. Control cells contained medium without $AlPcS_4$. Plates were incubated at 37 °C in 5 % CO_2 in the dark for 24 h, the wells were then washed twice with 2 ml DPBS and the medium replaced with 1 ml of culture medium. The loss of sensitizer from cells to medium was not observed.

Surviving cells were quantified after re-incubation with culture medium with the use of NR viability assay (Biochrom AG, Berlin, Germany) after 48 h. Cell survival was expressed as percentage of non-treated controls (0 µM $AlPcS_4$).

NR assay was used instead of the currently used viable assays like the commonly used yellow tetrazolium 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay for reasons of economy. However, Borenfreund and Puerner [18] have described the NR assay as a sensitive assay to evaluate viable cells in monolayer cultures.

Phototoxicity test of $AlPcS_4$

Phototoxicity of $AlPcS_4$ was determined by incubating the cells with 20 µM $AlPcS_4$ for 24 h. Cells were seeded into six 4-well tissue culture plates at a density of 4.8 x 10^5 cells/well in 1 ml of culture medium. Cells were allowed to attach for 48 h before being washed twice with 2 ml DPBS, then photosensitized by the addition of culture medium containing 20 µM $AlPcS_4$.

Control wells contained medium without $AlPcS_4$ and 0 J/cm^2 diode laser irradiation. Plates were incubated at 37 °C in 5 % CO_2 in the dark for 24 h, the wells were then washed twice with 2 ml DPBS and the medium replaced with 1 ml of culture medium without phenol red. Wells containing cells treated with $AlPcS_4$ were irradiated with a 676 nm diode laser (LM-100-670, Applied Optronics, South Plainfield, NJ, USA) with a power density of 100 mW/cm^2 and irradiation at 0.5–5 J/cm^2 for 10–100 s, respectively. One of the 4-well plates with cells untreated with $AlPcS_4$ was irradiated with 5 J/cm^2 and was used as additional control. The culture medium without phenol red was then replaced with 1 ml of fresh supplemented culture medium and the preparation returned to the incubator for further 48 h. Surviving cells were quantified after re-incubation with culture medium with the use of NR viability assay after 48 h. Cell survival was expressed as percentage of non-treated controls (0 µM $AIPcS_4$, no laser irradiation).

Measurement of absorbance

In both cytotoxicity and phototoxicity tests from each sample a 100 µl aliquot of lysed cells solution was transferred to a 96-well microtiter plate, and absorbance was measured at 570 nm on a multi-well reader (Lucy1; Anthos Labtec, Köln, Germany) using absorbance at 690 nm as reference whereby absorbance of NR from each sample is directly proportional to the number of living cells in the culture.

Laser scanning microscope

For each microscopic experiment, 10 glass bottom dishes (P35G-1.5-14-C; MatTeK, Ashland, MA, USA) were used. Four dishes were used for incubation with the sensitizer, and the other six were used for incubation with fluorophores for localization of lysosomes (LysoTracker® green), mitochondria (Rh-123) and marker of calcium inside the cell (Fluo-3). All three fluorescent probes were used at a final concentration of 1 µM. 50 cells per mm^2 were seeded on each glass bottom dish of 960 mm^2, 48 h before incubation with the sensitizer. The medium was removed and the cells were incubated for another 24 h with 20 µM $AlPcS_4$. The incubation period coincided with the growth phase of the HT-144 cells. The microscopic experiments were performed 40 min after removing the incubation medium and rinsing twice with PBS, replacing growth medium with DMEM without phenol red, supplemented with 10 % FCS. The cells in the dishes without $AlPcS_4$ were incubated with fluorophores for 40 min, respectively. Then for co-localization investigations, cells previously incubated with $AlPcS_4$ were further incubated for 40 min with the above probes, respectively.

The subcellular localization of the photosensitizers and the dynamic fluorescence changes during light exposure were evaluated using a LSM (Inverted LSM 510; Zeiss, Göttingen, Germany). A 63x magnification phase contrast objective lens (NA 1.2W corr.; Zeiss, Göttingen, Germany) was used together with a zoom factor of 1. After cells were incubated with 20 µM of $AlPcS_4$ for 24 h and washed with DPBS, the fluorescence imaging of cell samples was measured. The fluorescence of $AlPcS_4$ was excited with the HeNe laser at 633 nm and 10.5 µW and detected with the LSM 510 META detector (Zeiss, Göttingen, Germany) at 643–717 nm. It was assigned the color red. Fluorescence of probes Rh-123, LysoTracker® and Fluo-3 was excited with the argon ion laser at 488 nm (LGK 7786 P; LASOS, Jena, Germany) and detected in a channel using a bandpass filter (range: 500–550 nm). Different colors were assigned to this channel depending on the probe: Rh-123 was displayed in green, LysoTracker® in blue and Fluo-3 in sky-blue. Simultaneously, fluorescence of control cells, with or without molecular probes, was analyzed. Images were recorded in multitrack mode, i.e. one line with excitation at 633 nm and one line at 488 nm. Time series were recorded, either as stacks of up to 60 scans or as repeated series of stacks (3 x 25). The scanning time for single images was 6.7 s (25.6 µs per pixel) and for time series 3.35 s per image (12.8 µs per pixel) at a pixel size of 0.29 µm x 0.29 µm and an image size of 512 x 512 pixel. These parameters result in a dose of 0.134 nJ/pixel.

Results

Cytotoxicity of $AlPcS_4$

Cytotoxicity of $AlPcS_4$, as measured by quantifying surviving cells using the NR viability assay 48 h after the applied treatment, was found to be significant. Cytotoxicity tests showed that at 100 µM less than 40 % of the cells survived, and a slight sensitivity to 5, 10 and 20 µM with more than 60 % of survived cells (Fig. 1). The higher the concentration of $AlPcS_4$, the higher is the lost of cell viability of HT-144 cells.

Phototoxicity of $AlPcS_4$

Upon irradiation with diode laser at 676 nm, cells died in a concentration and light dose-dependent manner. The combinations of 20 µM $AlPcS_4$ and 1, 2, and 5 J/cm^2 light doses were phototoxic to the HT-144 cells.

At 20 µM $AlPcS_4$ and 5 J/cm^2 laser irradiation a more significant decrease in cell viability was observed using NR viability assays compared to the combinations of 20 µM $AlPcS_4$ with

1 and 2 J/cm^2 laser irradiation (Fig. 2). Viability studies have shown that the optimum phototoxic effect tested on HT-144 melanoma cells was obtained in the combination of laser dose of 1 J/cm^2 and 20 µM $AlPcS_4$. Student *t-test* was used to compare fluorescence intensity values of control cells and treated cells.

Laser scanning microscope

LSM was used to study the co-localization of $AlPcS_4$ with fluorescent cellular probes and its photodynamic effect in HT-144 cells. Excitation of $AlPcS_4$ fluorescence by the HeNe laser at 633 nm, according to the spectral characteristics of this photosensitizer as illustrated in Fig. 3, led to the fluorescence of $AlPcS_4$ which was therefore used to label the localization of $AlPcS_4$ in HT-144 cells. The HeNe laser reached a power density of 6 x 103 W/cm² at the sample and produced a PDT effect during repeated scanning. Heating of the sample can be neglected due to the small pixel size and short pixel time. The influence of the argon ion laser on the PDT effect could be neglected as $AlPcS_4$ has no absorption at 488 nm (Fig. 3).

Localization of cell organelles was observed using fluorescent probes, as shown in Fig. 4. Fluorescence of Rh-123, which was used to label active mitochondria, is displayed in green (Fig. 4a and b), while the distribution of lysosomes is evidenced by LysoTracker® green and displayed in blue (Fig. 4c and d). The Ca^{2+} marker Fluo-3 was used to visualize the cytoplasm and to observe Ca^{2+}-release upon irradiation. Fluorescence emission of this marker was assigned the sky-blue color (Fig. 4e and f). Fig. 4g shows the initial site of localization of $AlPcS_4$ in HT-144 cells at 24 h incubation. Localization of $AlPcS_4$ exhibited a diffuse pattern in the subcellular organelles of the HT-144 cells after incubation with 20 µM $AlPcS_4$ for 24 h (Fig. 4h). A small fraction of fluorescence of $AlPcS_4$ was seen in the nucleus or just below the membrane of the nucleus suggesting that some $AlPcS_4$ might be distributed in the cell nuclei (Fig. 5c).

When cells were co-incubated with $AlPcS_4$ and Rh-123, or LysoTracker®, the overlap of localization of $AlPcS_4$ and Rh-123 or rather $AlPcS_4$ and LysoTracker®, was observed. The localization pattern of $AlPcS_4$ corresponded very well with localization of the Rh-123 and LysoTracker®, but not with Fluo-3 at initial conditions as shown in Fig. 5c and Fig. 6. The combined fluorescence of $AlPcS_4$ and Rh-123 is shown by the yellow fluorescent spots (Fig. 5a) and $AlPcS_4$ and LysoTracker® by the purple fluorescent spots (Fig. 5b). With an increase in time of exposure of cells with $AlPcS_4$ to the HeNe laser light of the LSM at 0.8 mJ/cm^2, both an increase in fluorescence intensity of $AlPcS_4$ (Fig. 7, red line) in cytoplasm and a

redistribution of fluorescence localization from lysosomes into cytoplasm (Fig. 7, straight and broken red lines) were observed at higher light doses.

Redistribution of $AlPcS_4$ from subcellular localization into the cytoplasm was observed by an increase in fluorescence of $AlPcS_4$ in the cytoplasm (Fig. 6). Fluorescence intensity of the Ca^{2+}-marker Fluo-3 (Fig. 7, green line) increased after irradiation at 633 nm with 1.2 nJ, denoting a release of Ca^{2+} into the cytoplasm, presumably from mitochondria (Fig. 7, green line). The intensity shows a peak with a maximum at 2.3 nJ, returns to almost starting values after 3.35 nJ and increases again slowly until the end of the series. The apoptic bodies, blebbing of the membrane and shrinkage of the cells were noticed in response to photodamage of HT-144 cells with prolonged exposure to LSM laser light in the presence of $AlPcS_4$ (Fig. 8). These results suggest that $AlPcS_4$ can be used in PDT of skin melanoma cancers. $AlPcS_4$ has shown to cause damage of mitochondria and lysosomes leading to diffuse distribution of $AlPcS_4$ in the cytoplasm possibly with production of singlet oxygen in the cytoplasm.

Discussion

PDT is currently being accepted and established worldwide as the treatment destroys only the targeted tumor tissue whilst leaving normal tissue intact. The aim of this work was to evaluate $AlPcS_4$ for use as a suitable photosensitizer for PDT of skin melanoma cancers. One of the characteristics of an ideal photosensitizer is that it should have a strong absorption of light at longer wavelengths (600–800 nm) where tissue penetration of light is at maximum while still being energetic enough to produce singlet oxygen [5]. Our results revealed that $AlPcS_4$ absorbs light in the long-wave region of the visible part of the electromagnetic spectra (Fig. 3). Viability studies using NR viability assay have shown that the optimum phototoxic effect tested on HT-144 melanoma cells was obtained in the combination of a laser dose of 1 J/cm^2 and 20 μM $AlPcS_4$.

Current knowledge suggests that the success of PDT lies in the subcellular localization of the photosensitizer. The plasma membrane, nucleus, mitochondria and lysosomes have been identified as targets for photosensitizer localization [6]. Localization of photosensitizers in the cells plays a vital role in determining the mechanisms of cell death in PDT [15]. The $AlPcS_4$ was readily taken up by HT-144 cells in the 24 h of incubation (Fig. 4h). LSM using fluorescent probes was employed for detailed visualization of the localization of $AlPcS_4$ in melanoma cells (HT-144 cell line) in mitochondria and lysosomes. LSM is useful for its

ability to image thin optical sections of cells or living tissue, rejecting out-of-focus light and producing sharp images of every section [16]. In a previous study LSM was used to study the subcellular localization of $AlPcS_n$ in human melanoma cell lines which was found to be in the lysosomes [17]. However, our study revealed that $AlPcS_4$ is localized in both mitochondria and lysosomes (Fig. 2). This work is in agreement with the work of Tamietti et al. [19] who found that $AlPcS_4$ leads to significant alterations of target organelles of the cell during PDT by causing membrane potential loss. Our observations suggest that using LSM, destruction of cells during PDT can be monitored. Distribution of the $AlPcS_4$ inside the cytoplasm during LSM micrograph of cells with $AlPcS_4$ exposed to standard laser sources (argon ion laser: λ_{em}=488 nm; HeNe: λ_{em}=633 nm), suggests that during irradiation the photosensitizer from accumulated organelles leaks out of the damaged membranes of the target organelles leading to localization of $AlPcS_4$ in the cytoplasm. Simultaneously, a release of Ca^{2+} into the cytoplasm takes place, revealed by an increase in Fluo-3 fluorescence, and gives evidence for mitochondrial damage [20]. After 30 min of cells exposed to LSM, clear signs of apoptotic cell damage are visible (Fig. 8) up to a destruction of the whole tumor cell. Clearly, the cytoplasmic organelles are also important intracellular targets of $AlPcS_4$ during PDT; therefore they also play a crucial role in determining the cell death mechanisms of PDT. $AlPcS_4$ was shown to be localized in insignificant amounts in the nucleus (Fig. 6). These results are in good agreement with work reported in [17].

Conclusion

The results suggest that $AlPcS_4$ is a potential sensitizer for melanoma skin cancer treatments. However, a comparison of its cytotoxicity, uptake and accumulation in normal and cancer cells deserves further investigation.

Acknowledgements

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Zusammenfassung

Subzelluläre Co-Lokalisation von tetra-sulfoniertem Aluminium(III)-Phthalocyanin-Chlorid ($AlPcS_4$) mit Fluoreszenzmarkern in der humanen Melanom-Zelllinie HT-144

Hintergrund: Die Bestrahlung eines Photosensibilisators mit sichtbarem Licht, vorzugsweise Laserlicht im roten Spektralbereich, und in Gegenwart von molekularem Sauerstoff führt zu einer photochemischen Reaktion, die als photodynamische Therapie (PDT) bezeichnet wird. Die PDT wird zunehmend für die Behandlung von Hauttumoren eingesetzt. Für den Erfolg der PDT ist die subzelluläre Lokalisation des Photosensibilisators ausschlaggebend. In Mitochondrien lokalisierte Photosensibilisatoren können beschleunigt Apoptose auslösen. In Lysosomen lokalisierte Photosensibilisatoren können entweder Nekrose oder Apoptose hervorrufen. Die vorliegende Arbeit trägt zur Klassifikation subzellulärer Zielstrukturen bei, die über entsprechende Signalwege zum Zelltod führen.

Material und Methoden: Die Lokalisation von hydrophilem, tetra-sulfoniertem Aluminium(III)-Phthalocyanin-Chlorid ($AlPcS_4$) in metastasierenden humanen Melanom-Zellen (HT-144) wurde mit Hilfe von Fluoreszenzmarkern (Rhodamin 123, LysoTracker® und Fluo-3) und dem Laser-Scan-Mikroskop (LSM) untersucht.

Ergebnisse: Die Laserstrahlquellen im LSM erzeugten einen PDT-Effekt, der zu einer Umverteilung des $AlPcS_4$ in den Zellen führte, gefolgt von einem Fluoreszenzanstieg des $AlPcS_4$ im Zytoplasma der HT-144 Zellen.

Zusammenfassung: $AlPcS_4$ scheint ein potenter Photosensibilisator für metastasierende humane Melanom-Zellen (HT-144) zu sein; jedoch sind weitere vergleichende Messungen an gesunden Zellen notwendig.

Schlüsselwörter: Photosensiblisatoren; Photodynamische Therapie (PDT); Phthalocyanine; Co-Lokalisation; Laser-Scan-Mikroskop (LSM)

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Legends

Figure captions

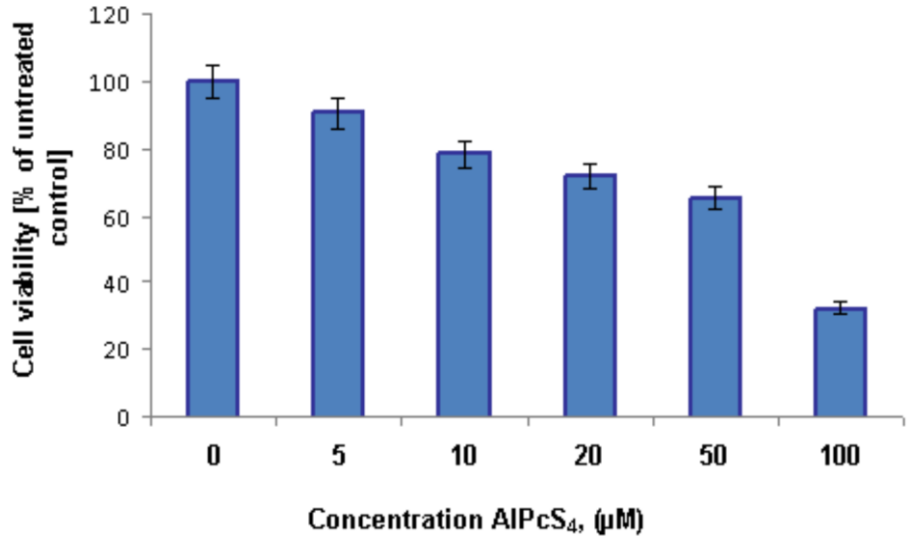

Fig. 1. Cytotoxicity of $AlPcS_4$. Untreated HT-144 cells (control; 0 μM of $AlPcS_4$) were compared with those treated with 5–100 μM of $AlPcS_4$.

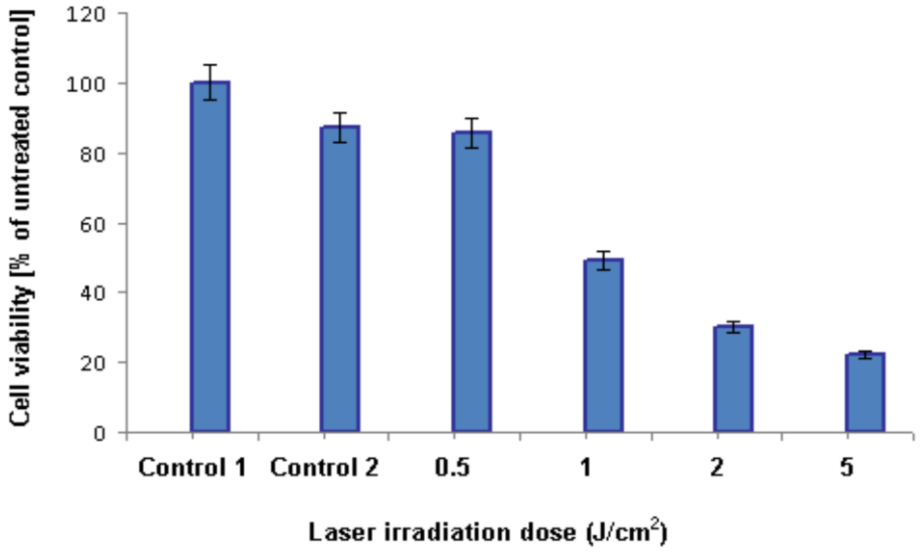

Fig. 2. Phototoxicity of $AlPcS_4$. Untreated HT-144 cells (without $AlPcS_4$ and without irradiation, control 1) were compared with those treated with 20 μM of $AlPcS_4$ and irradiated with 0.5–5 J/cm^2. Further control: HT-144 cells without $AlPcS_4$ but with irradiation of 5 J/cm^2 (control 2).

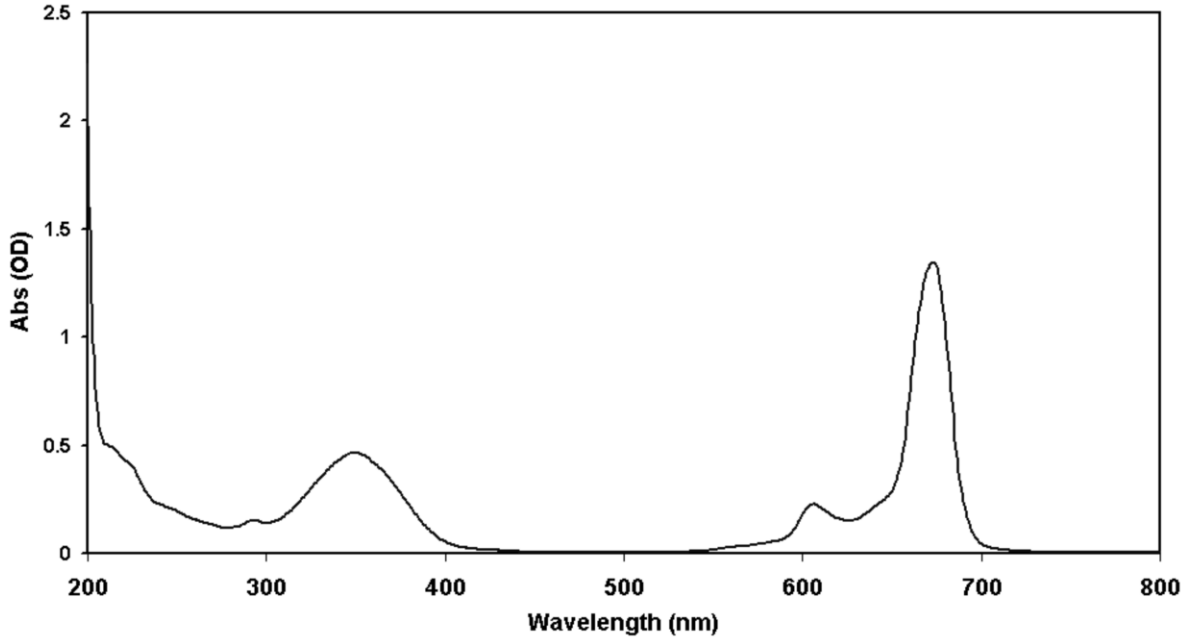

Fig. 3. Qualitative absorption spectra of $AlPcS_4$ in Dulbecco's phosphate balanced solution (DPBS). Absorption maximum of $AlPcS_4$ in DPBS solution can be seen at 673nm.

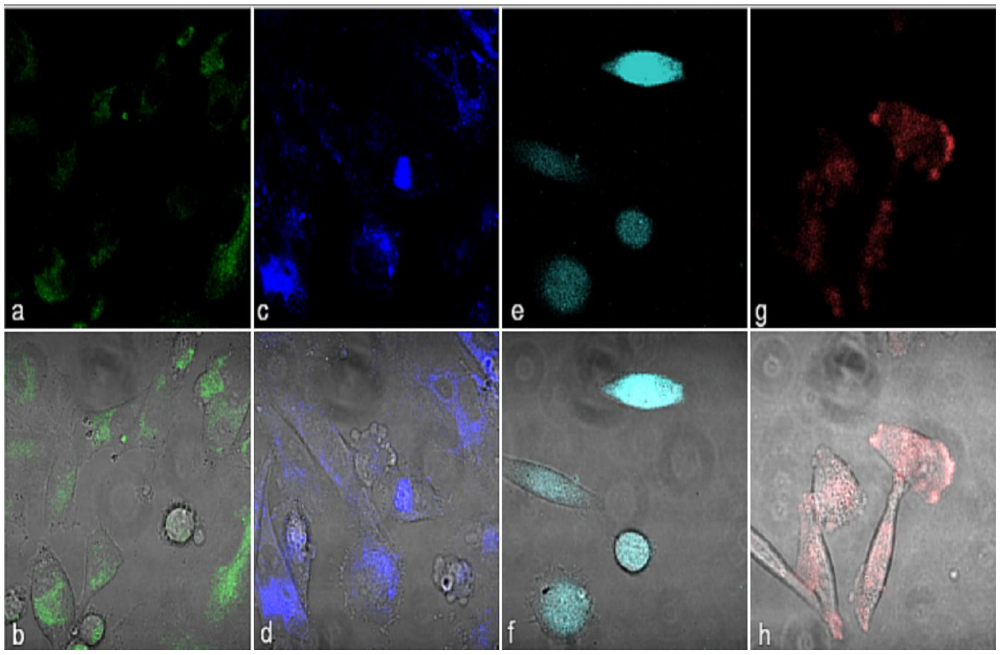

Fig. 4. Localization of subcellular fluorescent probes. Upper panels: fluorescence only; lower panels: superposition of fluorescence signal and phase contrast. (a) and (b): mitochondria; (c) and (d): lysosomes; (e) and (f): calcium in cytoplasm; (g) and (h): $AlPcS_4$.

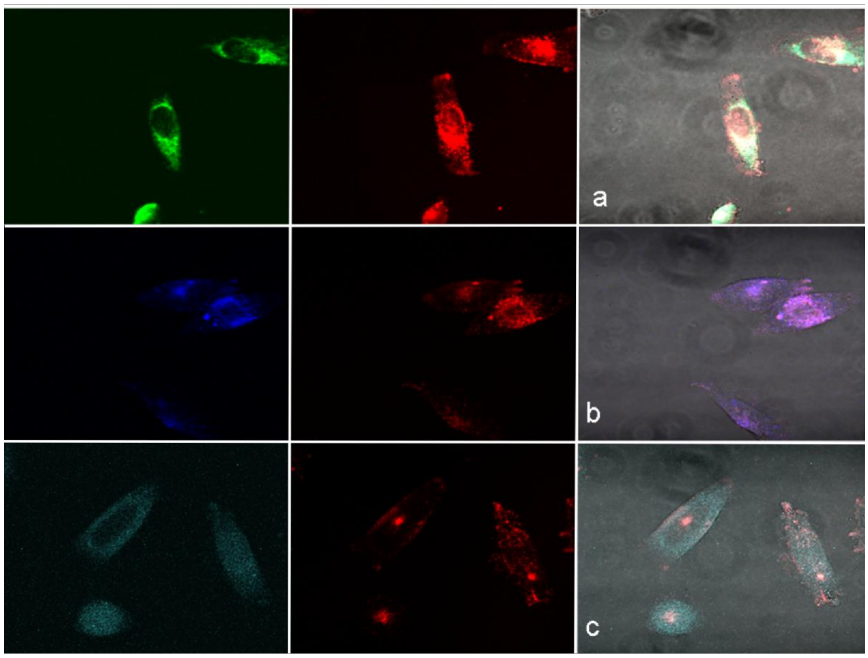

Fig. 5. Co-localization of fluorescence of (a) Rh-123 and $AlPcS_4$ and (b) LysoTracker® and $AlPcS_4$. (c) No initial co-localization of Fluo-3 and $AlPcS_4$. Left panel: cellular probe; middle: $AlPcS_4$; right: superposition.

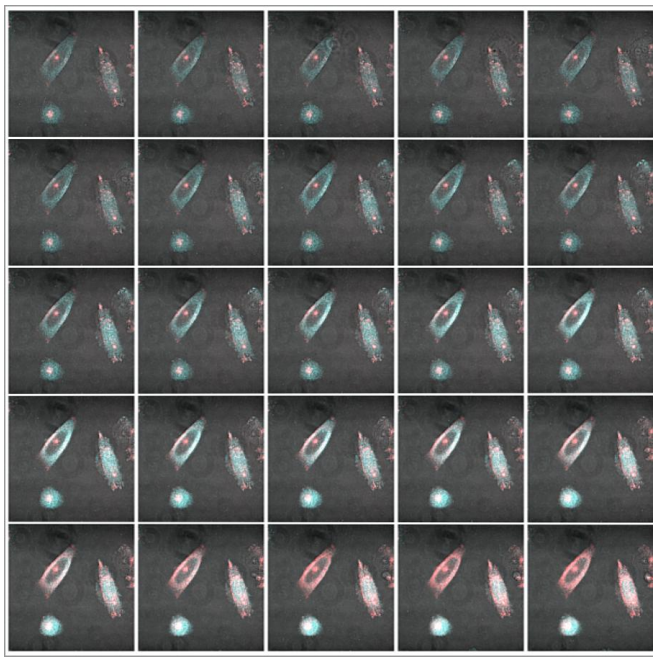

Fig. 6. Subcellular localization of $AlPcS_4$ (red), Fluo-3 (sky-blue) and distribution of $AlPcS_4$ in the cytoplasm as studied by LSM. Changes in fluorescence intensity of Fluo-3 are observed and a redistribution of $AlPcS_4$ with release into the cytoplasm. Scanning and irradiation at 633 nm and 10.5 µW, 25 frames at 3.35 s/frame; further parameters as in LSM section of 'Material and methods'.

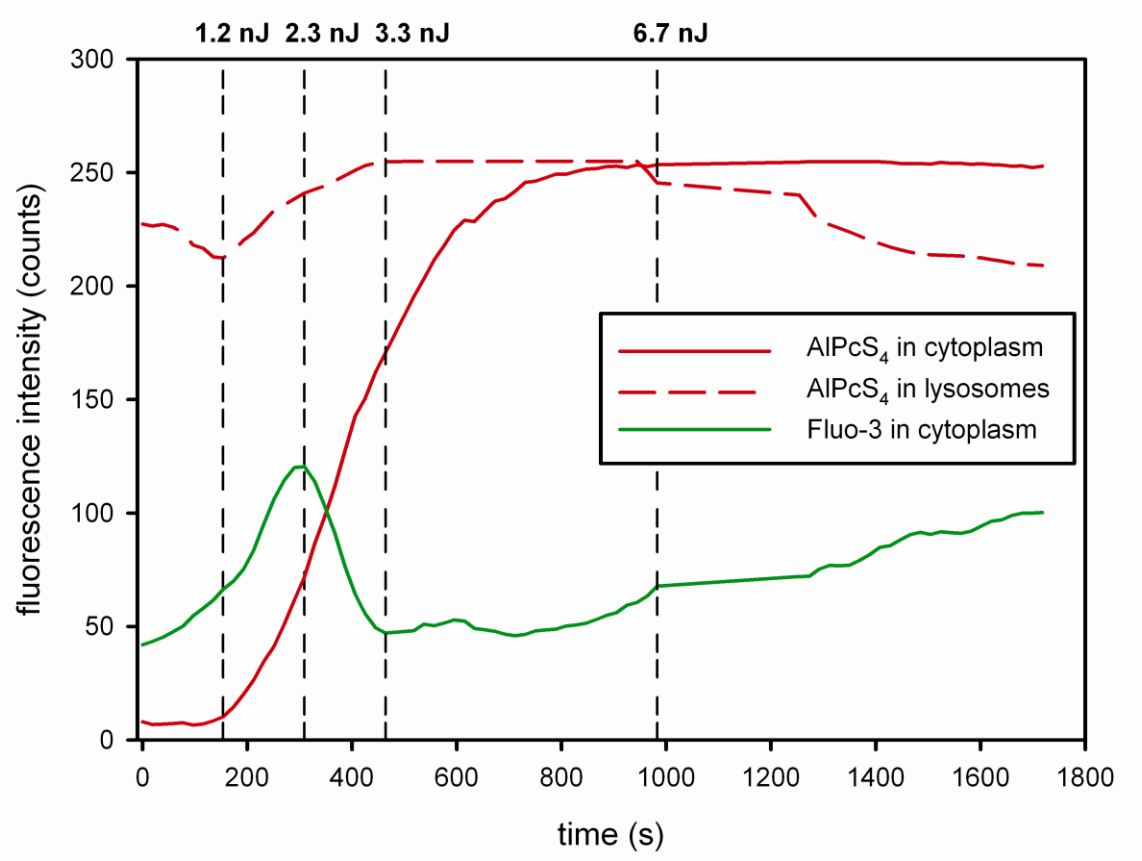

Fig. 7. Subcellular fluorescence of $AlPcS_4$ and Fluo-3 in HT-144 cells with an increase in time of light exposure. There is an increase of $AlPcS_4$ fluorescence in cytoplasm (red line); dose-dependent decrease of $AlPcS_4$ fluorescence in lysosomes and subsequent increase to cytoplasmatic level (red broken line) and dose-dependent increase of Fluo-3 fluorescence due to Ca^{2+} release into the cytoplasm (green line).

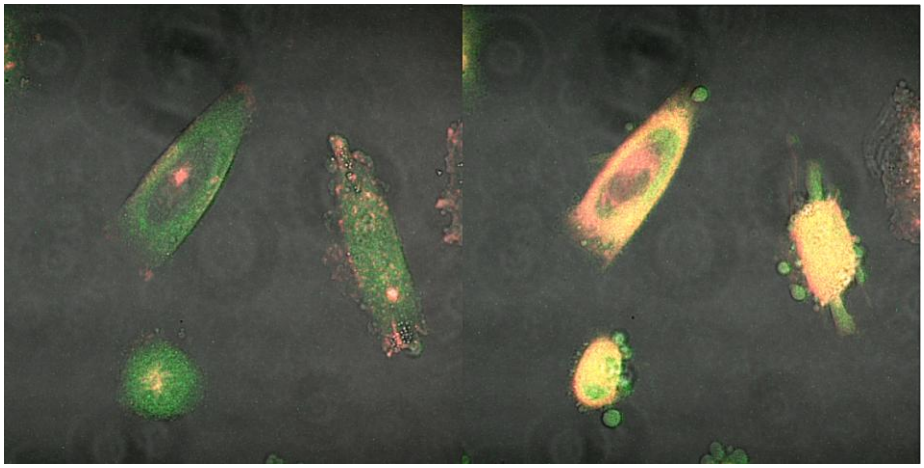

Fig. 8. Changes in fluorescence intensity and cell morphology due to laser irradiation: cell shrinkage, blebbing of membrane and formation of apoptotic bodies. Left: before irradiation; right: after irradiation at 633 nm with 0.8 mJ/cm^2. Cells were incubated for 24 h with 20 μM of $AlPcS_4$.