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Hypericin activated by an incoherent light source has photodynamic effects on esophageal cancer cells

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Abstract *Background and aims:* Photodynamic therapy (PDT) is a new treatment modality for early esophageal neoplasia. With two absorption maxima in the visible light range (550 and 588 nm) hypericin is a very promising photosensitizer for PDT with incoherent light sources. We studied the effects of photosensitizing hypericin in both primary cell cultures and cell lines (squamous: Kyse-140 and adenocarcinoma: OE-33) of human esophageal cancer using an incoherent white light source. *Materials and methods:* Esophageal cancer cells were preincubated (4–24 h) with hypericin (10 nM–1 μM) and then irradiated with a light energy dose of 30 J/cm^2. *Results:* Hypericin showed strong phototoxic effects and induced apoptosis in a dose-dependent fashion. The IC_{50} value of hypericin phototoxicity was approximately 30 nM in both squamous and adenocarcinoma cells. In the concentrations used nonphotoactivated hypericin showed no toxic or apoptotic potency. The phototoxicity of hypericin was compared to that of δ-aminolevulinic acid (5-ALA), which is already being used for photodynamic therapy of gastrointestinal cancer. 5-ALA produced similar phototoxic effects but at a much higher dose (IC_{50} 182± 8 μM in Kyse-140 and 308±40 μM in OE-33 cells). Moreover, 5-ALA did not induce apoptosis to a relevant extent. *Conclusion:* Hypericin is a very promising new photosensitizer for innovative photodynamic therapy of esophageal cancer. Both the well known clinical safety of hypericin and the lower costs of broad band light sources argue in favor of clinical trials.

Keywords Esophageal cancer · Photodynamic therapy · Hypericin · Primary cell culture · Apoptosis

Introduction

Endoscopic mucosal resection (EMR) [1] and photodynamic therapy (PDT) are effective treatment modalities for high-grade dysplasia or even early esophageal cancer [2]. Additionally, PDT has been used successfully to treat bile duct cancer [3], pancreatic cancer [4], colorectal cancer [5, 6], and gastric cancer [7]. PDT involves the activation of a photosensitizing drug by irradiation to locally generating reactive oxygen species (e.g. singlet oxygen and superoxide anion radicals) which induces tumor shrinkage by necrosis and/or apoptosis. The currently used photosensitizers, such as hematophorphyrin derivate and porfimer sodium, induce systemic photosensitization for up to 2 months and cause esophageal stricture formation in one-third of patients [8]. Therefore new photosensitizers must be evaluated for PDT of esophageal cancer.

Hypericin is a naturally occurring polycyclic aromatic naphthodianthrone that is isolated from plants of the *Hypericum* genus [9, 10]. The nonphotosensitized compound has no genotoxicity [11] or toxicity in animals or humans [12, 13]. Moreover, extracts of *Hypericum perforatum* are clinically safe drugs which have been used

for years as a popular herbal remedy for mild and moderate depression [14, 15, 16]. Photosensitized hypericin produces a high number of photosensitizing effects due to a high generation of singlet oxygen quantum [17] and superoxide anion radicals [18]. Hypericin has been shown to be effectively photoactivated by broad-band light sources with a spectral output of 400–700 nm, matching the dual absorption maximum of hypericin in the visible light range (550 and 588 nm) [19]. It qualifies as a new photosensitizer for PDT inasmuch as hypericin PDT induced cytotoxicity and apoptosis have been documented in various tumor models [20, 21]. Esophageal cancer still remains to be investigated in this respect.

The goal of this investigation was to evaluate hypericin photoactivated by an incoherent white light source for its effects on human squamous and adenocarcinoma cells of the esophagus. The possible clinical implications of our findings were also examined in primary cell cultures of biopsy specimens endoscopically obtained from esophageal cancer patients. The efficiency of PDT with hypericin was compared to that achieved with δ-aminolevulinic acid (5-ALA), which has been used successfully in PDT of esophageal cancer [22].

Materials and methods

Primary cell cultures

Isolated primary cell cultures were established from endoscopic biopsy specimens of histologically verified squamous esophageal carcinomas taken from seven esophageal cancer patients (five men, two women; mean age 62±6 years). The human tumor material was used according to the standards set by the Ethics Committee of the Benjamin Franklin University Hospital, Free University of Berlin. Mechanical dissection was performed with an automated disaggregation system (Medimachine; Becton Dickinson, Heidelberg, Germany) as described elsewhere in detail [23]. Cells were maintained in Earle's 199 medium (Biochrom, Berlin, Germany), containing 20% fetal calf serum, 2 mM L-glutamine, 2% (vol/vol) Biotect protective medium (Biochrom), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% vol/vol amphotericin B. Cells were kept in an incubator at 37°C in a humidified atmosphere of 5% CO_2. One-half the medium was changed every day. Cells remained in culture for at least 2 days before the experiments were carried out. Trypan blue exclusion tests revealed that the proportion of dead cells never exceeded 10%. Epithelial origin of the isolated tumor cells was confirmed by immunostaining with anti-cytokeratin antibody (clone MNF 116, Dako, Hamburg, Germany).

Cell lines

The human squamous esophageal carcinoma cell line Kyse-140 [24] and the human esophageal adenocarcinoma cell line OE-33 (ECACC ref. no. 96070808) were maintained in standard RPMI 1640 medium complemented with 10% fetal calf serum (Biochrom-Seromed, Berlin, Germany) and kept in an incubator (37°C, 5% CO_2, humidified atmosphere). Medium of OE-33 cells additionally contained 2 mM L-glutamine.

Light source and irradiation

The radiation unit used a 100-W halogen lamp (HLX 64627, Osram, Germany) as the light source. The spectral output of the lamp ranged from 400 to 800 nm. To prevent infrared irradiation, a heat-reflecting filter (Präzisions Glas & Optik, Iserlohn, Germany) that cuts off transmission at 700 nm and above was inserted into the optical path. The illuminated area (11×14 cm) had an average power density of 15 mW/cm^2. The light energy dose was measured with a P-9710 radiometer controlled by a silicon photocell (RW-3703-2 radiometer) from Gigahertz-Optik (Munich, Germany). The total light energy dose was calculated by integrating the energy signal over the entire period of irradiation. Cells were illuminated for 33 min to achieve a total light dose of 30 J/cm^2.

Uptake of photosensitizers and PDT treatment

Hypericin and 5-ALA were purchased from Sigma Chemicals (Deisenhofen, Germany). 5-ALA is an endogenous porphyrin precursor that stimulates the synthesis of protoporphyrin IX (PpIX), the active compound responsible for photodynamic tissue effects [25]. As with hypericin, PpIX also has an absorption peak in the visible light range (635 nm) and can therefore be effectively stimulated by the radiation unit used in this study [26]. The absorption characteristics of the hypericin used in this study were verified by spectral analyses which were kindly performed by the Institute of Laser Medicine, University Hospital Benjamin Franklin, Free University of Berlin.

To determine hypericin uptake, cells were incubated with rising hypericin concentrations (10 nM–1 µM hypericin) for 0–24 h. Cells were washed three times with phosphate-buffered sodium chloride solution (PBS) and intracellular hypericin fluorescence (590 nm) was measured with a FACScalibur flow cytometer (Becton Dickinson, San Jose, Calif., USA) or monitored with an Axioskop-2 fluorescence microscope (Zeiss, Jena, Germany). For PDT treatment cells were preincubated in the dark at 37°C (5% CO_2, humidified atmosphere) for 4–24 h with hypericin (10 nM–1 µM) or for 24 h with 5-ALA (100–500 µM) in culture medium containing 10% fetal calf serum. The medium was removed, and cells were irradiated in photosensitizer-free PBS for 33 min. During irradiation the temperature of the samples never exceeded 37°C, nor did it decrease below 33°C. After irradiation PBS was removed, and cells were maintained at 37°C (5% CO_2, humidified atmosphere) in photosensitizer-free culture medium. Dark controls were treated in the same way except irradiation.

Measurement of cell proliferation by bromodeoxyuridine assay

Cell proliferation was measured by a colorimetric immunoassay (Roche Diagnostics, Mannheim, Germany) based on bromodeoxyuridine (BrdU) incorporation during DNA synthesis. The assay was performed according to the manufacturer's recommendations as previously described [27]. After PDT treatment, samples and controls were cultured for up to 5 days in photosensitizer-free medium. At 24 h intervals BrdU-labeling solution was added for 4 h. After removal of the BrdU-labeling solution cells were fixed, denatured, and then incubated with a peroxidase-conjugated anti-BrdU antibody (anti-BrdU-POD). After washing off the unbound anti-BrdU-POD the color reaction was measured using a microplate reader.

Detection of changes in cell numbers by crystal violet assay

Changes in cell numbers were determined by measuring crystal-violet stained DNA contents of the samples and comparing them with those of untreated controls [28, 29]. Assays were performed

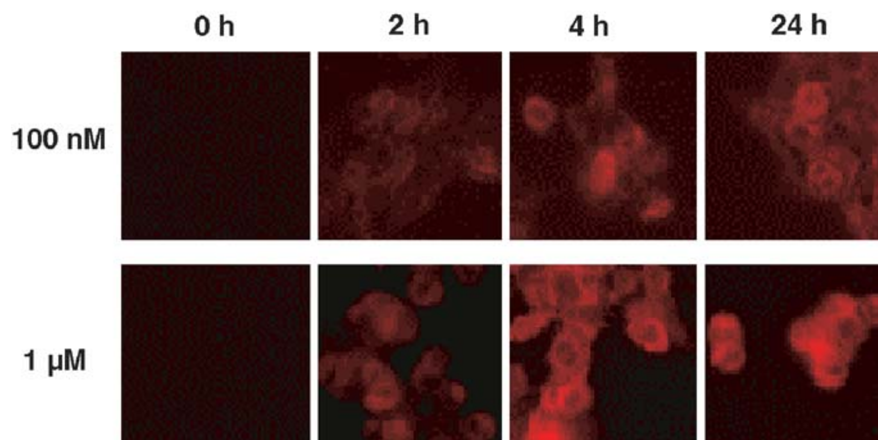

Fig. 1 Time- and dose-dependent hypericin uptake in primary cell cultures of squamous esophageal cancer. Hypericin uptake in primary cell cultures was determined by fluorescence microscopy after 0–24 h of incubation with either 100 nM (*upper panel*) or 1 µM hypericin (*lower panel*). Representative findings from seven independent experiments

in 96-well plates with 5,000 cells/well seeded at the beginning of the experiment. Each concentration group consisted of ten wells.

Determination of caspase-3 activity

After irradiation cells were maintained in photosensitizer-free culture medium in an incubator (37°C, 5% CO_2, humidified atmosphere) for 2–8 h. Cells were then washed twice with PBS and stored at –80°C until use. Approximately 10^6 cells were lysed with 500 µl lysis buffer (10 mM Tris-HCl, 10 mM NaH_2PO_4/Na_2HPO_4, 130 mM NaCl, 1% Triton-X-100, 10 mM $NaPP_i$, pH 7.5), and total protein content was quantified using a BCA protein assay kit (Pierce, Rockford Ill., USA). Caspase-3 activity was calculated from cleavage of the fluorogenic substrate DEVD-AMC [23, 24]. Therefore 100 µl cell lysate containing 500 µg/ml protein was incubated with 100 µl of substrate solution (2 µg caspase-3 substrate AC-DEVD-AMC, 20 mM HEPES, 10% glycerol, 2 mM DTT, pH 7.5) for 1 h at 37°C. The cleavage of DEVD-AMC was measured with VersaFluor fluorometer (Bio-Rad, Munich, Germany) using 360-nm excitation and 460-nm emission wavelength.

Cell death detection ELISA

A photometric enzyme-linked immunoassay (Cell death detection ELISA, Boehringer-Mannheim, Mannheim, Germany) was performed to detect DNA fragmentation. The test detects mono- and oligonucleosomes characteristic of apoptosis in the cytoplasmic fraction of cell lysates [27]. After irradiation cells were incubated in culture medium (37°C, 5% CO_2, humidified atmosphere) for 12–24 h. The cells were then washed twice with PBS and stored at –80°C until use. Approximately 10^6 cells were lysed with 500 µl lysis buffer (10 mM Tris-HCl, 10 mM NaH_2PO_4/Na_2HPO_4, 130 mM NaCl, 1% Triton-X-100, 10 mM $NaPP_i$, pH 7.5), and the total protein content was quantified using a BCA protein assay kit (Pierce). The ELISA was used as recommended by the manufacturer and described in detail elsewhere [30].

Determination of cell viability

Cell viability of irradiated esophageal carcinoma cells was measured with a cell viability/cytotoxicity assay kit (live/dead assay) from Molecular Probes (Leiden, The Netherlands) as described elsewhere in detail [27]. Cells grown on glass coverslips were incubated for at least 4 h with hypericin (10 nM–1 µM) or for 24 h with 5-ALA (50–500 µM). After washing off the incubation medium with PBS cells were irradiated as described above. PBS was then removed, and cells were incubated with calcein-AM (160 nM) and EthD-1 (2 µM) for 1 h in PBS at 37°C. Calcein (ex/em approx. 495/510 nm) and EthD-1 (ex/em approx. 495/635 nm) fluorescence was used for monitoring living and dead cells with a fluorescence microscope from Zeiss (Axioskop-2; Jena, Germany). Live and dead cells in each sample were quantified by calculating the average value of at least four arbitrarily chosen image areas of the respective coverslip.

Results

Time- and dose-dependent uptake of hypericin in esophageal cancer cells

Incubating esophageal cancer cells with rising hypericin concentrations (10 nM–1 µM) for 1–24 h led to a time- and dose-dependent intracellular accumulation of the dye. Flow cytometric analysis of Kyse-140 and OE-33 revealed that the dose-related intracellular accumulation of hypericin increased time-dependently during the first hours of incubation, reaching a stable and lasting plateau after 4 h. Increasing the incubation period up to 24 h did not further increase the intracellular fluorescence of the respective hypericin concentration (data not shown). Comparable results were obtained by fluorescence microscopy of hypericin-treated primary culture cells of squamous esophageal carcinomas (Fig. 1).

Evaluation of immediate phototoxicity of PDT with hypericin or 5-ALA

The toxicity of photoactivated hypericin and 5-ALA in primary cultures and cell lines of esophageal carcinomas was determined immediately after irradiation by simultaneous fluorescence labeling of viable (green) and dead (red) cells using live/dead assays. Previous cell survival studies after irradiation alone (in the absence of any photosensitizer) demonstrated no substantial differences from the dark controls (data not shown). Both hypericin

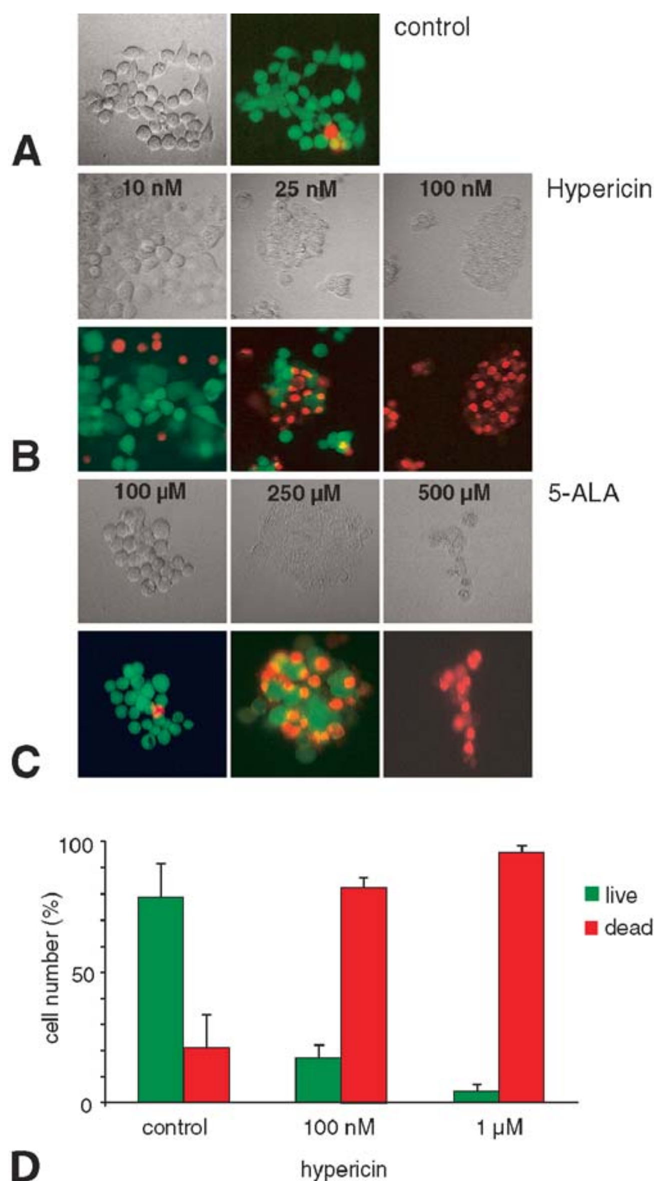

Fig. 2A–D Evaluation of immediate toxicity of photoactivated hypericin and 5-ALA in esophageal cancer cells. The phototoxicity of hypericin or 5-ALA was determined by live/dead assay immediately after PDT. **A** Control conditions of untreated (but irradiated) Kyse-140 cells. **B**, **C** Dose-dependent effect of photoactivated hypericin (**B**) and 5-ALA (**C**) on Kyse-140 cells. *Above* Phase-contrast images; *below* corresponding fluorescence micrographs. Representative findings of four independent experiments. **D** Phototoxicity of hypericin PDT (100 nM, 1 µM) in isolated primary cell cultures of human esophageal cancers. Means as percentage of viable versus dead cells ±SEM of five independent experiments are shown

and 5-ALA had dose-dependent phototoxic effects on esophageal squamous and adenocarcinoma cell lines. Compared to irradiated but untreated controls, irradiated cells pretreated with either hypericin or 5-ALA underwent dramatic morphological changes and appeared unstructured, shrunken, and flat (Fig. 2). Comparable results were obtained with hypericin PDT of primary squamous esophageal cancer cells: up to 96±3% of hypericin PDT treated cells died immediately after irradiation (Fig. 2D).

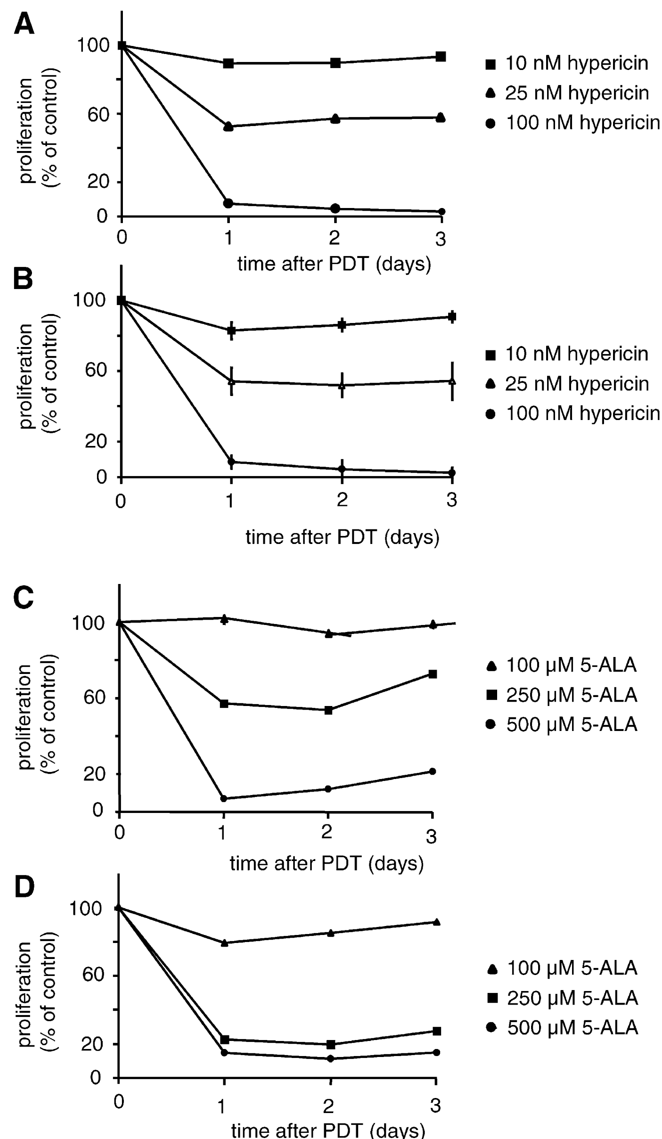

Fig. 3A–D Hypericin and 5-ALA PDT in esophageal cancer cells. Lasting decrease in cell numbers. Dose-dependent decrease in Kyse-140 cells (**A**, **C**) or OE-33 cells (**B**, **D**) after PDT with hypericin (**A**, **B**) or 5-ALA (**C**, **D**). Changes in cell number measured by crystal-violet assays are given as percentages of the untreated control. Means ±SEM of four to six independent experiments are shown

Lasting effects of PDT with hypericin or 5-ALA

Lasting effects of single PDT on cell survival of esophageal cancer cells of either histology (squamous or adeno-

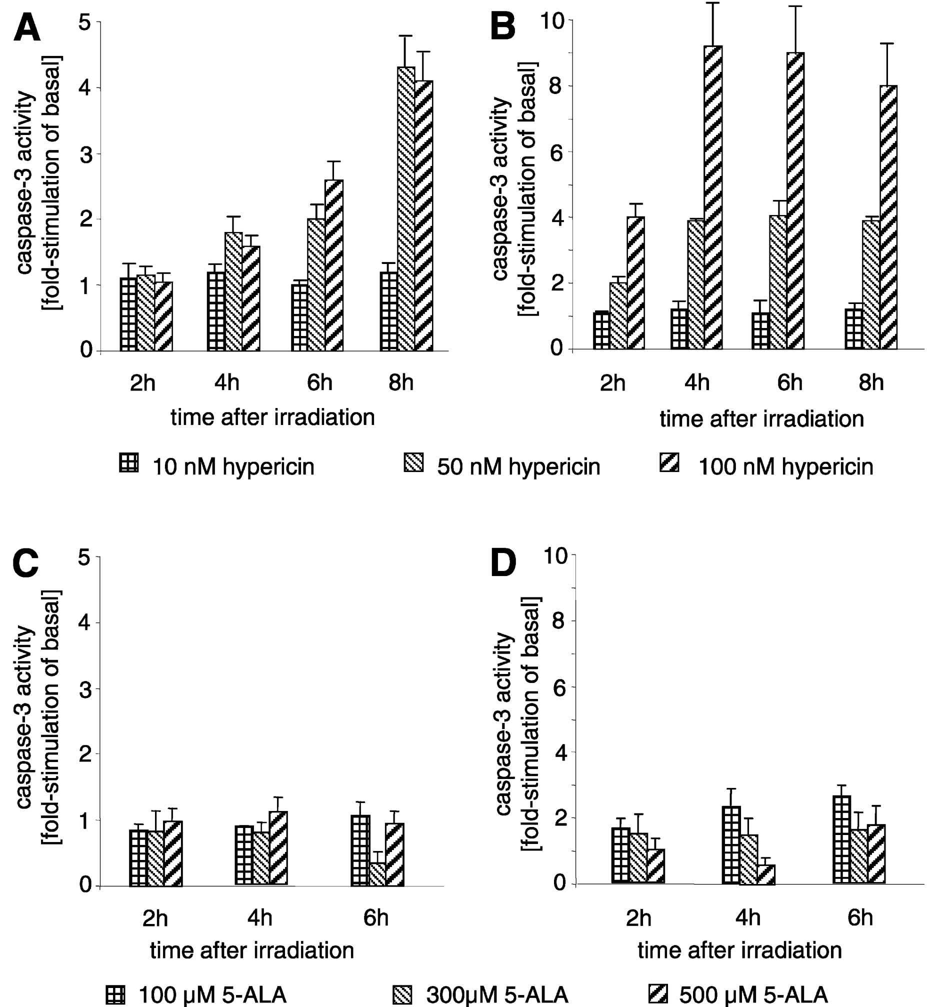

Fig. 4A–D Apoptosis-specific activation of caspase-3 in esophageal cancer cells after PDT with hypericin or 5-ALA. Caspase-3 activity was determined by measuring the cleavage of the fluorogenic substrate DEVD-AMC after PDT with hypericin (**A**, **B**) or 5-ALA (**C**, **D**) in Kyse-140 cells (**A**, **C**) or OE-33 cells (**B**, **D**). Means ±SEM of three independent experiments are given as percentage of control

carcinoma) were determined for up to 5 days after irradiation by performing crystal-violet assays and measuring BrdU incorporation into newly synthesized DNA of metabolically active cells.

Irradiation dose-dependently decreased the cell numbers of squamous Kyse-140 and adenocarcinomatous OE-33 cells pretreated with hypericin compared to untreated controls (Fig. 3A, 3B). Nonphotoactivated hypericin (10 nM–1 μM) did not affect cell proliferation of either Kyse-140 or OE-33 cells (data not shown). The IC_{50} value of hypericin phototoxicity calculated 1 day after PDT was 28±1 nM for Kyse-140 cells and 27±1 nM for OE-33 cells. No change or shift in the IC_{50} value was observed when the calculations were performed on day 2 or 3 after PDT. Both cell lines showed maximal effects with a decrease in cell number greater than 95% at hypericin concentrations of 100 nM. The fact that the number of cells in hypericin PDT treated samples had not reincreased even after 3 days indicated that there was no appreciable reproliferation. This finding was confirmed by proliferation assays measuring BrdU incorporation on day 1 and 5 after PDT. Kyse-140 or OE-33 cells which escaped from (≥50 nM) hypericin PDT induced phototoxicity did not show a significant BrdU incorporation 24 h after PDT or during the following 4 days (data not shown).

Single PDT dose-dependently decreased Kyse-140 and OE-33 cells pretreated with 5-ALA compared to the untreated controls (Fig. 3C, D). Without irradiation 5-ALA did not significantly decrease either Kyse-140 or OE-33 cells. However, 1 mM 5-ALA slightly but significantly decreased Kyse-140 cells by 9±2% compared to untreated controls, while proliferation of OE-33 cells remained unaffected even at this high concentration (data not shown). The IC_{50} value of photoactivated 5-ALA, specified 1 day after PDT, was 182±8 μM in Kyse-140 and 285±10 μM in OE-33 cells. Furthermore, a time-dependent shift in the IC_{50} value of 5-ALA PDT treated adenocarcinoma cells was observed. Three days after PDT the IC_{50} of 5-ALA for OE-33 cells had markedly increased to 308±40 μM.

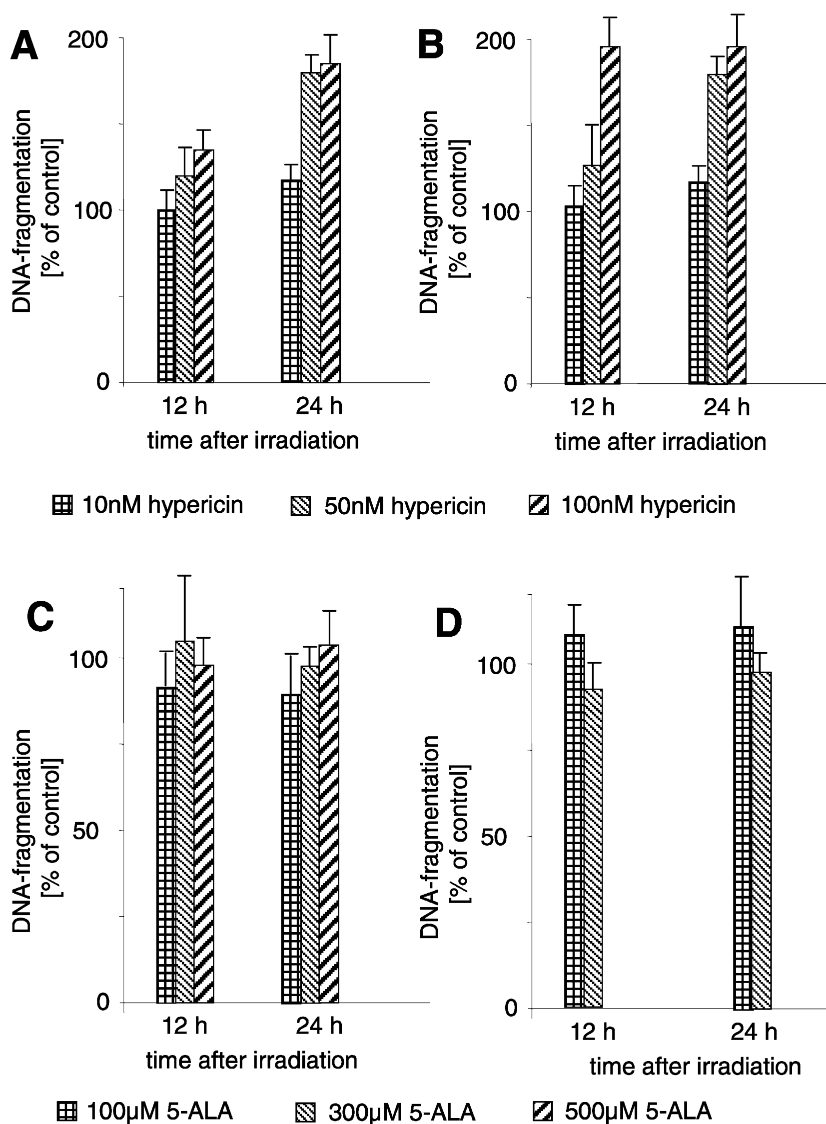

Fig. 5A–D Apoptosis-specific DNA fragmentation of esophageal cancer cells after PDT with hypericin or 5-ALA. DNA fragmentation of esophageal cancer cells was determined 12 and 24 h after PDT with hypericin or 5-ALA by performing cell death detection ELISAs. DNA fragmentation of Kyse-140 cells (**A**, **C**) or OE-33 cells (**B**, **D**) after PDT with hypericin (**A**, **B**) or 5-ALA (**C**, **D** is given as a percentage of the untreated control. Means ±SEM of three independent experiments are shown

Esophageal cancer cells that escaped from being killed by 5-ALA PDT started to grow again 3 days after PDT. BrdU assays revealed that immediate reproliferation of Kyse-140 cells occurred only with low 5-ALA concentrations (100–300 µM; data not shown). OE-33 cells which escaped from 5-ALA PDT induced phototoxicity showed no decrease in DNA-synthesizing activity at all. Even at 5-ALA concentrations greater than 500 µM the DNA-synthesizing activity of the remaining OE-33 cells on day 5 after PDT remained completely unaffected and did not differ from the untreated control cells (data not shown).

Apoptotic effects of photoactivated hypericin or 5-ALA

Induction of apoptosis in esophageal squamous or adenocarcinoma cells by photoactivated hypericin was determined by measuring caspase-3 activity and by assessing DNA fragmentation. Both Kyse-140 and OE-33 cells showed a time- and dose-dependent increase in caspase-3 activity (Fig. 4A, B) and an increase in apoptosis-specific DNA fragments (Fig. 5A, B). Dark controls with non-photoactivated hypericin (10–100 nM) did not exhibit any measurable increase in either capase-3 activity or DNA fragmentation (data not shown).

In contrast to the apoptosis-inducing effects of hypericin, after 5-ALA PDT the caspase-3 activity was nearly unaltered compared to the untreated controls in both cell lines (Fig. 4C, D). Even at the highest 5-ALA concentration (500 µM) caspase-3 activity was not increased 6 h after irradiation. In line with the lack of caspase-3 activation, no increase in apoptotic fragmentation of the DNA of Kyse-140 or OE-33 cells was disclosed by performing cell death detection ELISAs 12 and 24 h after 5-ALA PDT (Fig. 5C, D). Thus our data demonstrate

that 5-ALA did not induce apoptosis to any significant degree in either esophageal cancer type.

Discussion

In some Western countries the incidence of esophageal carcinoma has dramatically increased during the past two decades [31]. Although surgical resection with intent to cure is the treatment of choice for early carcinomas of the esophagus, the vast majority of patients ultimately requires palliative treatment [32]. PDT has proven to be an effective option for palliative treatment of advanced disease as well as for cure of Barrett's dysplasia and early cancers [2, 33]. State of the art is to use laser light for irradiation of photosensitizers such as hematoporphyrine derivatives or porfimer sodium. Photosensitizers of the first generation induce prolonged photosensitivity that can persist for up to 45 days after treatment [34, 35]. Moreover, hematoporphyrines are impure and unstable compounds and are thus far from being ideal photosensitizers [36].

Here we investigated the applicability of the naturally occurring plant pigment hypericin photoactivated by an incoherent light source as a new photosensitizer for PDT. It has been shown that hypericin is a powerful drug for innovative PDT treatment of tumors of the bladder, glioblastomas, and fibrosarcomas [37, 38, 39]. Uptake studies in patients with flat bladder carcinoma have demonstrated that in vivo hypericin preferably accumulates in the tumor. However, the exact mode of this intensified accumulation remains to be clarified [40].

Our present study on esophageal cancer cells confirms that hypericin activated by visible light is a potent and effective photosensitizer for treating both squamous and adenocarcinoma cells of the esophagus. Photoactivated hypericin proved to be both highly cytotoxic and antiproliferative. Hypericin-PDT even induced apoptosis at low nanomolar concentrations. The present data substantiate earlier findings obtained by using hypericin for PDT in other cancers [41, 42, 43, 44]. Moreover, experiments with primary cell cultures of human esophageal squamous cell cancers confirmed the data obtained in the esophageal cell lines OE-33 and Kyse-140, thus suggesting that hypericin-PDT may well gain clinical relevance in the future. Since (nonphotoactivated) hypericin has been widely used for treating mild and moderate depressions, its clinical safety has been well documented [45, 46].

The findings on hypericin were compared to those obtained with 5-ALA PDT treated esophageal cancer cells. We observed that the concentrations of 5-ALA needed to produce toxic effects in esophageal cancer cells were much higher than those of hypericin. Moreover, the IC_{50} values of 5-ALA differed between the two esophageal cancer histologies, while those for hypericin did not, indicating a more general applicability of hypericin as photosensitizer for PDT of esophageal neoplasia. Furthermore, we did not observe significant apoptosis induced by photoactivated 5-ALA in either squamous or adenocarcinoma esophageal cancer cells. Thus hypericin-based PDT appears to be a promising alternative to the yet used photosensitizers. Especially the induction of apoptosis by hypercin PDT is advantageous since the suicidal cell program leads to an inflammation free elimination of cancer cells thereby avoiding unwanted side effects of the treatment.

Another interesting feature of hypericin became apparent in the studies on reproliferation of cells surviving PDT. While hypericin-treated esophageal cancer cells which escaped from being killed by PDT did not reproliferate for up to 5 days after irradiation, 5-ALA-treated squamous cell and adenocarcinoma cells which escaped from immediate 5-ALA phototoxicity started to regrow instantaneously. The mechanism underlying the hypericin PDT induced proliferation change in surviving cells was not further investigated in this study. However, when studying the antiproliferative effects of hypericin in glioma cells, Miccoli et al. [47] showed that photoactivation of hypericin leads to an intracellular pH decrease accompanied by an alteration in mitochondrial-bound hexokinase. This initiates a cascade of events that alters the glucose energy metabolism and survival of glioma cells. Thus it is conceivable that hypericin exerts its antiproliferative actions on PDT-surviving esophageal cancer cells by an intracellular pH decrease that ultimately impairs the mitochondria-dependent glucose energy metabolism. Further studies are necessary to clarify whether pH-dependent destruction of mitochondrial energy metabolism also occurs in hypericin PDT treated esophageal cancer cells and to determine whether this mechanism is causative for the observed antiproliferative effects. In the meantime, the very fact that an antiproliferative effect does exist is of great significance even though the underlying mechanisms still remain unknown. It implies that PDT with hypericin induces long-lasting antineoplastic effects which are of clinical relevance as concerns future PDT strategies in the management of esophageal neoplasia. For clinical use of hypericin PDT special catheters must be engineered as to apply the required light intensity in a controlled manner at the sites of interest. Patients suffering from multifocal intraepithelial neoplasia [48] may be candidates for PDT with systemically administered hypericin.

Our study performed photosensitization using an incoherent light source instead of the commonly employed laser light. This was chosen since the use of laser light has some disadvantages. The spectrum of laser light is essentially monochromatic, which is not ideal for photosensitizers with several absorption maxima and excludes combining photosensitizers with different absorption maxima. Finally, lasers are more commonly used to treat

small areas, although magnifications of the treated area are possible to a certain extent by using diffusing fiber delivery systems. The drawbacks of lasers can be overcome by new photosensitizers effectively activated by nonlaser light sources with a broad spectral output. The applied light source should meet several requirements for photoactivated hypericin to induce an optimal yield of phototoxicity. It must match the absorption of the photosensitizer and have sufficient beam quality and power to deliver the necessary power to the treated cells. Thus PDT with hypericin can best be performed applying either monochromatic laser irradiation or nonlaser irradiation with a light source including the absorption peaks of hypericin at 550 and 588 nm. To impede unwanted tissue damage and to avoid heating effects UV and infrared radiation should be excluded. The broad band light source used in this study was developed in accordance with the above specific demands for PDT with hypericin. The halogen lamp based system had a spectral output of 400–700 nm, which included the dual absorption maxima of hypericin in the visible light range (550 and 588 nm). Infrared radiation was excluded by a heat-reflecting filter. Additional cooling by blowers ensured constant temperatures during irradiation.

The use of broad band visible light for irradiation offers several advantages. First, it allows the activation of cocktails of photosensitizers requiring different activation wavelengths. This could be of interest in future combinations of hypericin and first-generation photosensitizers [35]. Second, broad-band light sources are easier to apply and less expensive than lasers, especially if photosensitizer cocktails are favored. Third, broad-band light sources can be used to irradiate large surfaces. Interestingly, nonlaser light sources have already been applied in the endoscopic therapy of esophageal cancer [21].

We conclude that hypericin activated by an incoherent light source is a very promising new approach for innovative photodynamic therapy of intraepithelial esophageal neoplasia and merits further studies.

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