



Vitamin E (α -tocopherol) enhances the PDT action of hematoporphyrin derivatives on cervical cancer cells

El-Sayed A.M. Al-Sherbini^{a,*}, Amr H. El Noury^a, Mahmoud N. El Rouby^b, Tarek Ibrahim^a

^aNational Institute of Laser Enhanced Science (NILES), Cairo University, Cairo, Egypt

^bNational Cancer Institute (NCI), Cairo University, Cairo, Egypt

Received 29 September 2008; accepted 3 November 2008

Abstract

Objective: The effect of antioxidant vitamin E (VE) on PDT (a mainly reactive oxygen species-driven process) has in the past shown contradicting results. Hence, we studied the effect of different concentrations and different incubation periods of VE on the PDT cytotoxicity of the cervical adenocarcinoma HeLa cell line.

Materials and methods: HeLa cells were incubated with 25 μ g/ml of hematoporphyrin derivatives (HpD) for 25 min (1st PDT regimen) or 24 h (2nd PDT regimen), then irradiated with visible light (total light dose of 10 J/cm²) either with or without different concentrations of VE (1–1000 μ M) which had been incubated with the cells for 1 h or 24 h prior to PDT. After irradiation, viability was measured using MTT assay.

Results: The results obtained showed that PDT is effective against cervical cancer cells. Incubation of HpD for 24 h leads to improved PDT action. Higher concentrations of VE incubated in HeLa cells for 1 h before the 2nd PDT regimen significantly enhanced cytotoxicity of PDT and the maximal enhancement was at 1000 μ M of VE. The cytotoxic effect of VE on HeLa cells after incubation for 24 h before PDT is enhanced by the PDT action.

Conclusion: In conclusion 1000 μ M of VE can be used 1 h before PDT to enhance its effect on cervical adenocarcinoma, a disease which is steadily increasing in young women. It is well-known that the cervical adenocarcinoma is resistant to anticancer agents and radiotherapy and it was previously considered to be HpD-PDT resistant.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: HeLa cells; Cervical cancer; Photodynamic therapy (PDT); Vitamin E (α -tocopherol); Antioxidant

Introduction

Each of the traditional cancer therapy procedures (surgery, radiation, and chemotherapy) has its own limitations. As a result additional innovative approaches

must be developed for the treatment of human cancer. Photodynamic therapy (PDT) is a novel treatment for cancer, and for certain non-cancerous diseases that are generally characterized by overgrowth of unwanted or abnormal cells. PDT kills cancer cells by photochemical generation of reactive oxygen species following absorption of visible light by photosensitizers. These photosensitizers are selectively accumulated in tumors and characterized by a high quantum yield to generate high

*Corresponding author. Tel./fax: +20 2 5729499.

E-mail address: elsayed_s2000@yahoo.com
(E.-S.A.M. Al-Sherbini).

reactive singlet oxygen (cytotoxic oxygen) only in the presence of coherent and incoherent light [1]. To enhance the efficiency of PDT, several approaches have been considered. Combinations of PDT with local hyperthermia, ionizing radiation, radio sensitizers of the hypoxic cells, recombinant human tumor necrosis α -factor, or certain chemotherapeutic drugs such as Adriamycin and Mitomycin C have been studied [2]. Furthermore, results on the PDT-enhancing activities of certain antioxidant molecules such as α -tocopherol, Trolox[®] (a water soluble vitamin E analogue), ascorbate and 2(3)-tert-butyl-4-hydroxyanisole (BHA) have been reported to lead to improved tumor cell response. Different sensitizers and different tumor models used in these studies suggest that the observed phenomenon is not limited to a particular system [3].

Previous studies on the effect of vitamin E (VE) on PDT showed contradictory results; some showed that it protects against PDT cytotoxicity [4–7], while others found that VE enhances the PDT action or has no effect [8–10]. We have observed that there are differences between these studies concerning the PDT protocols used, as well as the incubation periods and concentrations of the vitamin.

Potential advantages of PDT for cervical cancer over conventional treatments such as surgery, cryotherapy, carbon dioxide laser ablation and electrosurgical excision are that it could eliminate intraepithelial lesions without causing profuse bleeding, vaginal discharge or a change in the location of the squamocolumnar junction. It could also spare young women with cervical neoplasm from conization which may cause adhesion and other side effects. It is also possible that large or multifocal lesions, or lesions that extend into the endocervical canal could be targeted through selective drug uptake whilst sparing adjacent normal cervical tissue. It seems that PDT could become an ideal option for treatment of gynecological lesions and tumors due to high response with the added benefit of potentially cosmetic results and organ preservation [11].

In our study, two different PDT regimens were investigated according to the incubation periods of hematoporphyrin derivatives (HpD). These are the short incubation period of HpD (25 min) where most HpD is found in the membrane, and the long incubation period (24 h) where HpD is spread across the cytoplasm and cell organelles. We also studied the effect of incubation of different concentrations of VE for 1 and 24 h on the PDT cytotoxicity of the cervical adenocarcinoma HeLa cell line.

Materials and methods

All chemicals were obtained from Sigma (USA) unless otherwise indicated.

Cell line

The human cervical carcinoma cell line (HeLa) was obtained from VACSERA, Cairo, Egypt. Cells were grown in RPMI-1640 medium containing 10% fetal calf serum (FCS) and antibiotics maintained in a humidified atmosphere of 5% CO₂ in air at 37 °C. Logarithmically growing cells were used for all experiments.

Preparation of HpD

Hematoporphyrin derivatives (HpD) were obtained from hematoporphyrin derivative dihydrochloride (Aldrich, Germany). A stock solution of HpD was prepared by dissolving 672 mg of HpD in 100 ml of glycerol and stirring overnight and the stock solution was kept in the dark. Working solutions of HpD (25 μ g/ml) were prepared immediately before use by further dilution of the stock solution with medium without serum. The working solution was sterile filtered using a 0.22 μ m micropore filter [12].

Preparation of VE concentrations

Vitamin E (α -tocopherol) was dissolved in a minimal amount of ethanol (final concentration of the ethanol in the concentrations used is <1% of the maximum concentration used in the study). The final concentration of ethanol after dilution with media was added separately to a group of untreated controls and no difference was found between untreated and vehicle treated controls.

For incubation of cultured cells, α -tocopherol from an alcoholic stock solution was pre-incubated with FCS and kept under dark conditions in the refrigerator. Immediately before use, the stock solution was diluted in serum-free medium to the specified concentrations (1, 10, 100 and 1000 μ M) and sterile filtered through a 0.22 μ m micropore filter before being added to the culture medium [13].

Photodynamic treatment

HeLa cells were harvested in the exponential phase of growth (using trypsin-EDTA) and suspended in RPMI-1640 medium with 10% FCS at a density of 40,000 cells/well (2×10^5 cells/ml). Cells were cultured in 96-well microtiter plates and incubated for 24 h for adhesion. After adhesion of cells, 25 μ g/ml HpD was added to each well (except untreated controls) and incubated in the dark at 37 °C in a humidified CO₂ incubator for 25 min (1st PDT regimen) or 24 h (2nd PDT regimen). Fresh phenol red-free RPMI-1640 medium, without FCS, was added to each well before irradiation.

Cells were irradiated with light source by a 500 W quartz halogen lamp at a distance of 60 cm from the plate. The infrared radiation was removed by a 4 cm

transparent water bag. We used a suitable filter to pass maximum light at wavelength of 630 nm.

The following measuring protocols were used to investigate the influence of different irradiation doses, HpD incubation times and VE concentrations on the HeLa cell viability:

A1: Effect of different irradiation doses

HeLa cells incubated with 25 µg/ml HpD for 24 h were exposed to different doses of light (1–15 J/cm²) as measured by a calibrated three channel Eldonet Dosimeter (Germany). Cell viability was measured 1 h (t1) after irradiation.

A2: Effect of different HpD incubation times

HeLa cells were incubated with 25 µg/ml of HpD, except untreated controls, for 25 min (1st PDT regimen) or 24 h (2nd PDT regimen). Cells were then irradiated with a light dose of 10 J/cm² (except dark controls). Cell viability was measured 1 h (t1) and 24 h (t2) after irradiation.

B1: Effect of vitamin E alone (dependent on VE incubation times and VE concentrations)

HeLa cells were incubated with different concentrations of VE only for 24, 48, and 96 h. Cell viability was evaluated by MTT assay at the end of the incubation period.

B2: Effect of vitamin E in combination with light irradiation

HeLa cells, pre-incubated with VE alone for 24 h, were irradiated with light at 10 J/cm². Cell viability was evaluated 24 h post-irradiation by MTT assay.

C1–4: VE-enhanced PDT

The two PDT regimens, differing in the length of the incubation period of HpD (25 min or 24 h), were combined with two protocols each differing in VE incubation periods (1 h or 24 h) so that we investigated four protocols as illustrated in Table 1.

For the application of the 1st PDT regimen, HeLa cells were incubated with different concentrations of VE (1–1000 µM) for 1 or 24 h in the dark, and then HpD

was added to the plates for the last 25 min of the incubation time.

For the application of the 2nd PDT regimen, HeLa cells were incubated with HpD for 24 h, and then different concentrations of VE (1–1000 µM) were added for 1 h or the HeLa cells were incubated with both HpD and different concentrations of VE for 24 h in dark.

In all protocols C1–C4, the cells were washed three times after incubation with normal sterile saline. Fresh medium without serum was added to the cells then irradiated with a total light dose of 10 J/m². Cell viability was measured 1 h (t1) and 24 h (t2) after irradiation.

Measurement of cell viability

Cell survival was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay (Sigma USA), which provides data equivalent to the clonogenic assay. Briefly, MTT 5 mg/ml was dissolved in RPMI-1640 medium without phenol red. This solution was sterile filtered through a 0.22 µm filter and stored at 2–8 °C for frequent use or frozen for extended periods. After 1 h (t1) or 24 h (t2) post-irradiation, 10 µl MTT stock solution was added to 200 µl fresh medium without serum to each well and incubated for 3–4 h at 37 °C to allow MTT metabolization. After incubation, the medium was removed and the formazan crystals were dissolved by adding 200 µl DMSO during shaking for 15–30 min. Absorbance of converted dye was measured at 570 nm by means of an ELISA plate reader (Metertech model E 960, Taiwan) [9,10].

Statistical analysis

Results were analyzed statistically using Student's *t*-test. Values of $p < 0.05$ were considered statistically significant. All data in the text and tables are expressed as a percentage of dark control \pm standard error (SEM) of at least three samples; experiments were repeated 3 or 4 times. The statistical analyses were carried out by GraphPad[®] Prism software (USA).

Results

A1: Effect of different irradiation doses on HeLa cell viability

Fig. 1 shows a considerable decrease in viability with an increase of the light dose. As shown in the figure, the light dose corresponding to the decrease in viability by approximately 50% (46.21%, $p < 0.001$) was 10 J/cm².

Table 1. VE-enhanced PDT.

Protocol No.	1st PDT regimen		2nd PDT regimen	
	C1	C2	C3	C4
Incubation time of HpD	25 min	24 h	24 h	24 h
Incubation time of VE	1 h	24 h	1 h	24 h

Differences in incubation time of HpD and VE.

A2: Effect of different HpD incubation times on HeLa cell viability

The effect of HpD was studied in combination with light irradiation on viability of HeLa cells after HpD incubation for 25 min (1st PDT regimen) and 24 h (2nd PDT regimen) and the results are illustrated in Table 2. The results obtained indicate that a longer HpD incubation period of 24 h is a more efficient in the PDT of HeLa cells.

B1: Effect of vitamin E alone on HeLa cells

Effects of incubation were studied for different concentrations of VE for 24, 48 and 96 h on HeLa cell line. The results illustrate that the incubation of HeLa cells with VE lead to a significant decrease in the

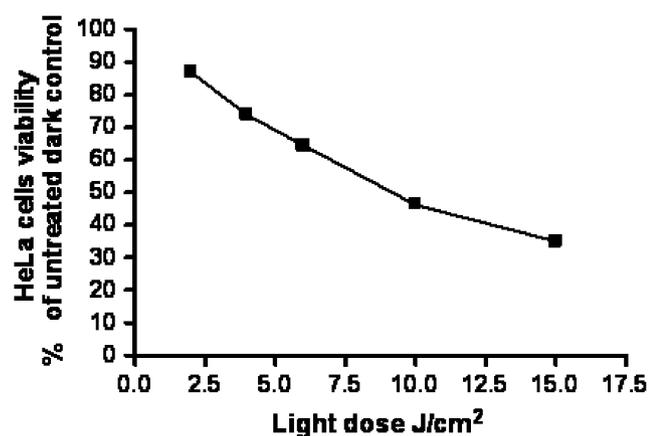


Fig. 1. The effect of different light doses on HeLa cells incubated for 24 h with 25 µg/ml HpD as measured by MTT assay 1 h after irradiation; data expressed as % of untreated dark control.

viability of the cells with increasing concentrations (Fig. 2). The highest decrease of viability was at 1000 µM. In addition, the results showed that the highest cytotoxicity was after 24 h of VE incubation. The cells showed some resistance to cytotoxic action at higher concentrations of VE, especially 100 µM after 48 and 96 h of incubation.

B2: Effect of vitamin E in combination with light irradiation

Photoirradiation of HeLa cells, pre-incubated with VE alone for 24 h, using 10 J/cm² causes a slight decrease in the viability of cells comparable to that induced by light alone on cells (data not shown).

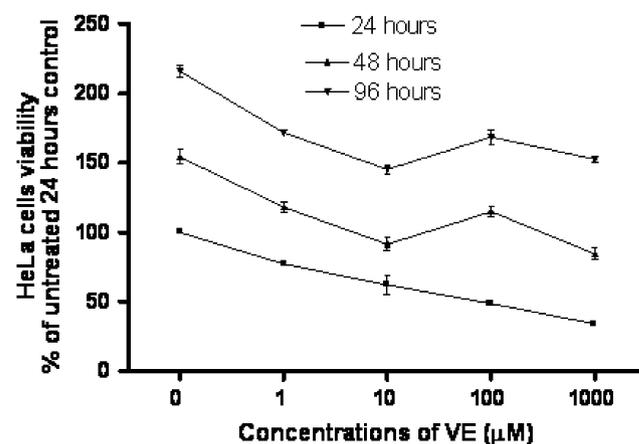


Fig. 2. Effect of VE incubation on HeLa cells with different VE concentrations for 24, 48, and 96 h as measured by MTT assay, considering the untreated control group after 24 h as 100%.

Table 2. Effect of incubation of HeLa cells with HpD followed by light irradiation of 10 J/cm².

Treatment	Viability after 1 h (t1)		Viability after 24 h (t2)	
	% of dark control	±SEM	% of dark control	±SEM
<i>Control groups</i>				
Untreated dark control	100.00	4.10	100.00	6.23
Cells only incubated with HpD for 24 h	96.42	6.04	94.30*	2.49
Cells irradiated with light only	97.23	1.85	95.35	3.24
<i>PDT groups</i>				
1st PDT regimen (25 min HpD incubation time)	73.74*	6.55	31.26**	2.34
2nd PDT regimen (24 h HpD incubation time)	46.21**	4.34	21.07***	2.41

Cell viability measured by MTT assay as percentage of untreated dark control.

p-Value is obtained as compared to untreated control group.

*Significant p-value ($p < 0.05$).

**Significant p-value ($p < 0.001$).

***Significant p-value ($p < 0.0001$).

C: VE-enhanced PDT

Comparison of the both PDT regimens showed that a slightly dark toxicity was observed only after a longer incubation period of HpD of 24 h (Fig. 3).

C1–2: First PDT regimen (HpD incubation for 25 min)

Results of the incubation of different concentrations of VE for 1 h (protocol# C1) and 24 h (protocol# C2) before the application of the 1st PDT regimen differ significantly between the two protocols.

Incubation of VE for 1 h showed that all VE concentrations decrease the viability of cells 1 h post-irradiation but have no effect 24 h post-irradiation, with the exception of 1 μM VE which seems to slightly protect the cells (Fig. 4A).

Incubation of VE for 24 h showed that all VE concentrations protect against cytotoxicity of PDT after 1 h (the observed decrease is comparable to that of the vitamin alone) and only 10 and 1000 μM VE enhanced the cytotoxicity of PDT 24 h post-irradiation (Fig. 4B).

C3–4: Second PDT regimen (HpD incubation for 24 h)

Incubation of VE for 1 h before the application of the 2nd PDT regimen (protocol# C3) showed that all concentrations of VE increase the cytotoxicity of PDT in a concentration dependant manner. The highest decrease in viability was at 1000 μM VE (Fig. 5A).

Incubation of VE for 24 h (protocol# C4) showed that all concentrations used protect against PDT action 1 h post-PDT and only vitamin-dependent cytotoxic action is observed. However, this protection was lost

24 h post-PDT. The highest concentration of VE, which was 1000 μM , increases the cytotoxicity of PDT (Fig. 5B).

Discussion

Cervical cancer is the third most commonly occurring cancer in women worldwide. It is a leading cause of cancer-related death and mortality in women in under-developed countries. Among the various histological types of cervical carcinoma, cervical adenocarcinoma is the one known to be resistant to anticancer agents and radiotherapy. Furthermore cervical adenocarcinoma (HeLa cells) have been found to be PDT resistant using a form of HpD (Photogem, Russia) at any dose, and to have a little dose-response only at 5 $\mu\text{g}/\text{ml}$, and light dose of 2 J/cm^2 [14,15].

The mechanisms underlying photodynamic action in cells and tissues are complex and depend upon multiple factors. Uptake and intracellular distribution of the photosensitizer, in addition to its photophysical and photochemical characteristics, are important in determining the efficacy of PDT. Studies showed that most of the HpD fluorescence was observed in the cell membrane after 30 min of incubation. However after 1–2 h the red fluorescence had increased in the cytoplasm and in the perinuclear region. It has previously been demonstrated that prolonged (>18 h) incubation with Photofrin[®] (which is a purified form of HpD) results in sub-cellular localization to the cytoplasmic organelles, including the mitochondria [10], while the Golgi complex is affected after 24 h incubation. These

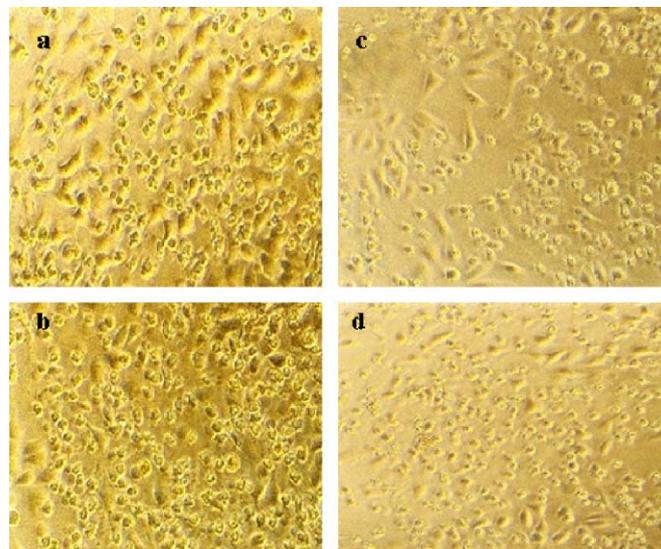


Fig. 3. Effect of VE incubation with 1000 μM VE on PDT of HeLa cells (see protocol# C3, 25 $\mu\text{g}/\text{ml}$ HpD for 24 h, total light dose: 10 J/cm^2). Comparison of PDT alone: HeLa cells 1 h (a) and 24 h (b) post-irradiation versus VE-enhanced PDT: HeLa cells 1 h (c) and 24 h (d) post-irradiation.

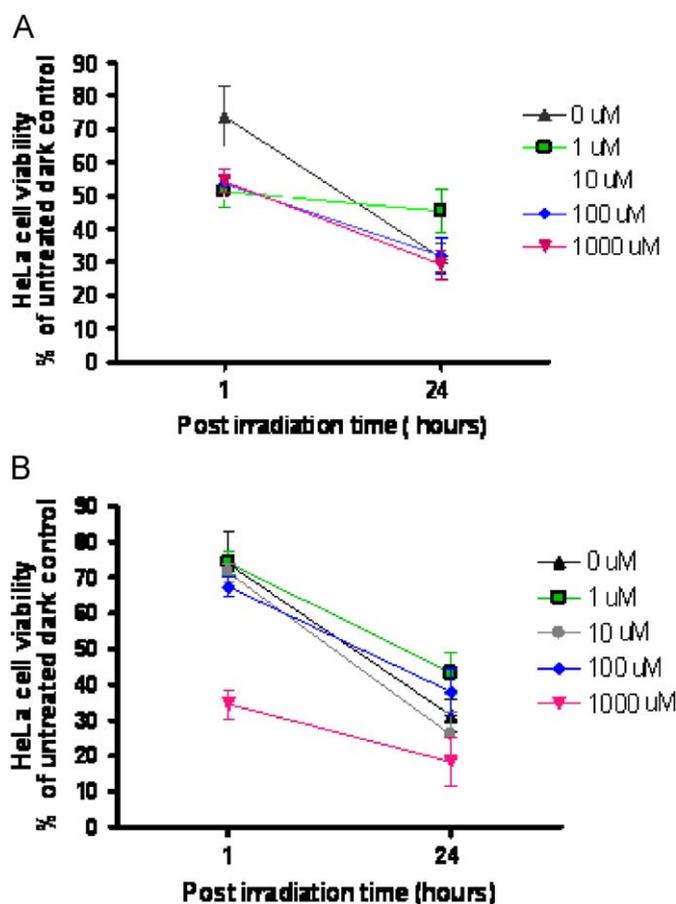


Fig. 4. The effect of incubation of HeLa cells with VE for 1 h (A – protocol# C1) and 24 h (B – protocol# C2) before the application of the 1st PDT regimen (25 $\mu\text{g}/\text{ml}$ HpD for 25 min, total light dose: 10 J/cm^2). Viability was measured 1 and 24 h post-irradiation.

porphyrins accumulate mainly via passive diffusion in cells, and are known to be localized in the cytomembranous structures such as mitochondria, lysosomes, endoplasmic reticulum, etc. giving diffuse fluorescence in the cytoplasm [16]. The effects of PDT on cells with plasma membrane-localized Photofrin[®] was found to be a cessation of proliferation post-PDT with Photofrin[®] doses less than 7 $\mu\text{g}/\text{ml}$. At higher doses (28 $\mu\text{g}/\text{ml}$), plasma membrane disruption and cell swelling were observed immediately after PDT [1].

In our study we studied the effect of HpD-PDT on HeLa cervical carcinoma cell lines. Two PDT regimens were investigated; in the 1st PDT regimen we incubated the cells with HpD for 25 min where it was localized mainly in the cell membrane. In the 2nd PDT regimen we incubated the cells with HpD for 24 h and it spread through cytoplasm and cellular organelles. We found that PDT is effective against the HeLa cell line in both regimens at a concentration of 25 $\mu\text{g}/\text{ml}$ HpD and a total light dose of 10 J/cm^2 . The localization of HpD in the 2nd PDT regimen of incubation (24 h) leads to improved PDT action. We used this high concentration of HpD (25 $\mu\text{g}/\text{ml}$) because at this concentration the main PDT

action leads to membrane disruption which can be theoretically prevented by VE (chain-breaking antioxidant).

Cells contain a large number of antioxidants to prevent, or repair the damage caused by reactive oxygen species. These include a number of small molecular antioxidants such as glutathione, vitamins E, C and A, as well as three types of larger molecular primary antioxidant proteins such as superoxide dismutase (SOD), catalase and glutathione peroxidase. Extensive evidence has shown that redox balance is impaired in cancer cells compared with normal cells. Altered levels of antioxidant enzymes such as SOD, catalase, glutathione peroxidase and non-enzymatic antioxidants such as glutathione (GSH), vitamin C, and thioredoxin, as well as changes in the related signal pathways, are evident in many human cancers. Antioxidant enzyme levels are variable in most animal and human cancers, but often higher in human tumors compared to normal tissue [9,17].

In our study we found that VE (α -tocopherol) alone significantly decreased the viability of HeLa cells, especially after 24 h of incubation, in a concentration

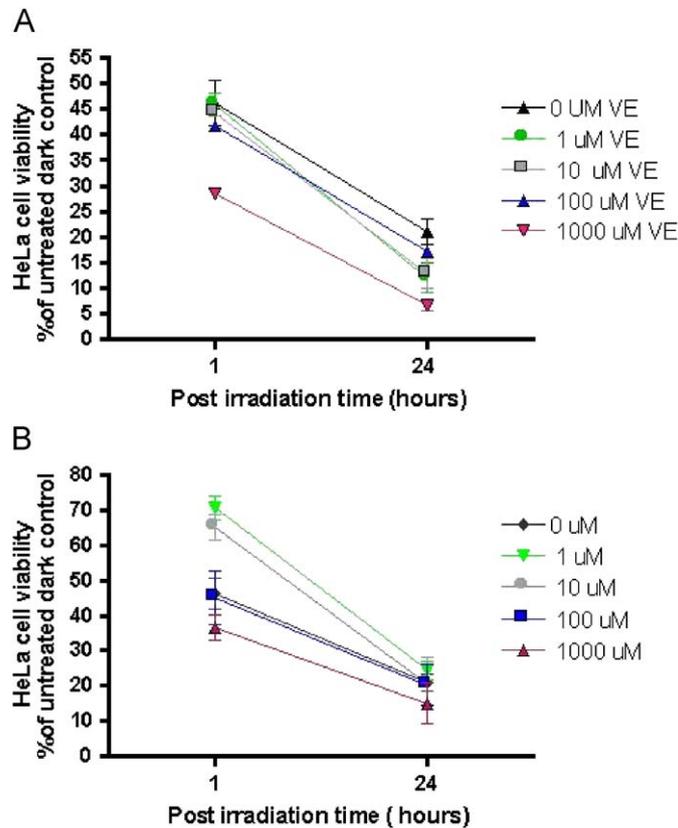


Fig. 5. The effect of incubation of HeLa cells with VE for 1 h (A – protocol# C3) and 24 h (B – protocol# C4) before the application of the 2nd PDT system ($25 \mu\text{g/ml}$ HpD for 24 h, total light dose: 10 J/cm^2). Viability was measured 1 and 24 h post-irradiation.

dependent manner. These results are in accordance with another study [9] which revealed that at a concentration of $1000 \mu\text{M}$, α -tocopherol was toxic to tumor HT29 cells (also adenocarcinoma) and non-toxic to normal cells (MRC-5 fibroblasts). For longer incubation periods at 48 and 96 h, cells became slightly resistant to the effect of VE especially at high concentrations. This has been observed in many human cancers which have become resistant to chemotherapeutic drugs or after an initial partial response [18].

Tumor responses following VE treatment might be due to its ability to inhibit tumor cell growth mediated by reactions resulting in reduction of important transcription factors and inhibition of cell response to growth factors. Furthermore, VE activates anti-tumor factors, including tumor necrosis factors ($\text{TNF-}\alpha$ and $\text{TNF-}\beta$), wild-type p53 and direct effects on tumor cells such as control of tumor growth through induction of differentiation and cell cycle inhibition [9,19]. Vitamin E and its derivatives have also shown potent apoptotic effects in several human cancer cell lines. It can inhibit tumor survival factors such as protein kinase C (PKC), which is a well-established anti-apoptotic factor expressed in several tumor types [20–22].

Some antioxidants act in a hydrophilic environment, others in a hydrophobic environment, and some act in both environments in the cell. For example, vitamin C reacts with superoxide in the aqueous phase while VE does so in the lipophilic phase. α -Tocopherol is the most active form of VE in humans and is a powerful biological antioxidant which is considered to be the major membrane-bound antioxidant employed by the cell. Its main antioxidant function is protection against lipid peroxidation [17]. VE may need time to be present in the membrane lipophilic phase where it has this effect as an antioxidant. This may explain the inability of VE to protect against PDT action after 1 h of vitamin incubation (protocol# C1 and C3). The difference between these two protocols is that during the 1st PDT regimen (HpD incubation for 25 min) the main PDT action is restricted to the cell membrane, so lower concentrations of VE protect against further destruction or prolonged action of PDT especially after 24 h post-irradiation (protocol# C1, Fig. 4A). During the 2nd PDT regimen HpD spreads into the cytoplasm and cell organelles, so VE does not protect against PDT but enhances the PDT action (protocol# C3, Fig. 5A).

On the other hand, when VE is incubated for 24 h before irradiation (protocol# C2 and C4) it protects

against PDT cytotoxic action immediately post-irradiation and the observed cytotoxicity is comparable to the cytotoxic action of VE alone. However, 24 h post-irradiation PDT enhances the cytotoxic effect of the vitamin in the cells and decreases their resistance towards the vitamin's cytotoxic action (Figs. 4B and 5B). One of the explanations for such an effect is that induction or inhibition of cell proliferation seems to be dependent on levels of oxidants/antioxidants in the cell. An enhanced reducing environment of the cell stimulates proliferation and a slight shift towards a mildly oxidizing environment initiates cell differentiation. A further shift towards a more highly oxidizing environment in the cell leads to apoptosis and necrosis. Apoptosis is induced by moderate oxidizing stimuli and necrosis by an intense oxidizing effect. Furthermore, there is a link between increased levels of reactive oxygen species (ROS) and disturbed activities of enzymatic and non-enzymatic antioxidants in tumor cells [17]. In addition, both forms of VE (tocotrienols and tocopherols) are oxidized as a result of free radical scavenging to corresponding quinones, which are prooxidants. It appears that the differences in the response of VE between normal tissue and cancer tissue are due to the inability of the cancer tissue to detoxify the prooxidant quinones which, if they accumulate in the tumor, can initiate new peroxidation locales and increase the lipid peroxidation of the tumor tissue. Thus, the preferential sensitization of tumor tissues to lipid peroxidation in presence of VE appears to be due to the unmetabolized prooxidant products of VE [23]. The prooxidant character of some antioxidants depends on the concentration and environment (oxygen pressure) in which they act [17]. α -Tocopherol has been reported to exert prooxidant activity both *in vitro* and *in vivo* at increased concentrations [9]. VE has also been shown to affect the activation of membrane signaling proteins such as protein kinase C via non-antioxidant behavior. Other non-antioxidant mechanisms include apoptosis by different mechanisms and inhibition of cell proliferation. The observed inability of VE to substantially prevent cell lyses could be due to the fact that it is consumed rapidly as a result of overwhelming lipid peroxidation during PDT, leading to membrane breakdown [5,6,8,10,24].

Another possible reason for the toxicity of reducing agents can arise from reaction with photodynamic sensitizer in its excited triplet state (3S), that leads to the formation of antioxidant and sensitizer radicals, therefore promoting type I photodynamic reactions. This reaction has been proposed for electron donors such as ascorbate, cysteine or reduced pyridine nucleotides, but cannot be theoretically excluded for other reducing agents [9].

The most striking result here is the action of $100\ \mu\text{M}$ of VE, which seems a critical concentration exerting a balance between prooxidant and antioxidant character-

istic of VE (note the strange behavior of this concentration in Figs. 2, 4 and 5).

The importance of this result arises from the recent studies which have reported that the incidence of invasive cervical adenocarcinoma, which used to account for only 10–15% of all cervical cancers, has been steadily on the increase in young women, despite the fact that the overall incidence of cervical cancer has declined. The cause of the increase is unclear, but it is of concern because studies have shown a poorer prognosis for patients with cervical adenocarcinoma than for those with squamous cell carcinoma [25]. PDT using a hematoporphyrin derivative photosensitizer is used clinically in the field of gynecology for the treatment of dysplasia and carcinoma in situ of the uterine cervix. However, cervical adenocarcinoma (HeLa cells) was found by another study [14] to be PDT resistant to HpD (Photogem, Russia) but according to our results PDT is effective against HeLa cervical adenocarcinoma cells and $1000\ \mu\text{M}$ of VE can be used to enhance the PDT action.

In conclusion, high concentrations of VE enhance the PDT action against HeLa cervical cancer cell line, the most effective protocol is the protocol# C3 where HpD was incubated for 24 h and VE incubated for only one hour before PDT.

Zusammenfassung

Effektivitätssteigerung der Photodynamischen Therapie mit Hematoporphyrin-Derivaten (HpD) bei zervikalen Krebszellen durch Vitamin E

Motivation: Der Einfluss des antioxidativ wirkenden Vitamins E (VE) auf die Photodynamische Therapie (PDT) – ein photooxidative Reaktionen verursachender Prozess – wurde in der Vergangenheit kontrovers diskutiert. Ziel der vorgestellten experimentellen Studie war es, den Effekt unterschiedlicher VE-Konzentrationen und -Inkubationszeiten auf die Zytotoxizität der PDT von HeLa-Zellen (humane Zelllinie des zervikalen Adenokarzinoms) zu untersuchen.

Material und Methoden: HeLa-Zellen wurden für 25 min (PDT-Modus 1) oder 24 h (PDT-Modus 2) mit $25\ \mu\text{g/ml}$ Hematoporphyrin-Derivat (HpD) inkubiert und anschließend mit sichtbarem Licht bestrahlt (Wellenlänge: 630 nm, Lichtdosis: $10\ \text{J/cm}^2$). Die PDT wurde allein oder in Kombination mit Vitamin E durchgeführt. Für die Kombinationstherapie wurden die HeLa-Zellen 1 h bzw. 24 h vor der PDT mit unterschiedlichen VE-Konzentrationen ($1\text{--}1000\ \mu\text{M}$) inkubiert. Nach der Bestrahlung wurde die Zahl der überlebenden Zellen mittels MTT-Assay gemessen.

Ergebnisse: Die Ergebnisse haben gezeigt, dass die PDT an den untersuchten zervikalen Krebszellen erfolgreich

war, wobei sich der zweite PDT-Modus mit einer HpD-Inkubationszeit von 24 h als effektiver erwies. In diesem PDT-Modus verbesserte zudem eine vorhergehende 1stündige Inkubation mit hohen VE-Konzentrationen die Zytotoxizität der PDT signifikant. Das beste Ergebnis für diese Kombinationstherapie wurde mit einer VE-Konzentration von 1000 μ M erreicht.

Zusammenfassung: Die Ergebnisse lassen den Schluss zu, dass die Gabe von 1000 μ M Vitamin E 1 h vor der PDT den Effekt der Therapie beim zervikalen Adenokarzinom – einem Tumortyp der zunehmend bei jungen Frauen diagnostiziert wird und von dem bekannt ist, dass er therapieresistent gegenüber Chemotherapie und Bestrahlung ist – verbessern kann. Bislang ging man davon aus, dass eine PDT mit HpD ebenfalls unwirksam ist.

Schlüsselwörter: HeLa-Zelllinie; Zervikales Adenokarzinom; Photodynamische Therapie (PDT); Vitamin E (α -Tocopherol); Antioxidant

References

- [1] Macdonald IJ, Dougherty TJ. Basic principles of photodynamic therapy: a review. *J Porphyrins Phthalocyanines* 2001;5(2):105–29.
- [2] Shevchuk I, Chekulayev V, Chekulayeva L. Enhancement of the efficiency of photodynamic therapy of tumours by t-butyl-4-hydroxyanisole. *J Photochem Photobiol B* 1998;45(2–3):136–43.
- [3] Jakus J, Farkas O. Photosensitizers and antioxidants: a way to new drugs? *Photochem Photobiol Sci* 2005;4(9):694–8.
- [4] Lin F, Girotti AW. Photodynamic action of merocyanine 540 on leukemia cells: iron-stimulated lipid peroxidation and cell killing. *Arch Biochem Biophys* 1993;300(2):714–23.
- [5] Geiger PG, Korytowski W, Lin F, Girotti AW. Lipid peroxidation in photodynamically stressed mammalian cells: use of cholesterol hydroperoxides as mechanistic reporters. *Free Radic Biol Med* 1997;23(1):57–68.
- [6] Zhang J, Cao EH, Li JF, Zhang TC, Ma WJ. Photodynamic effects of hypocrellin A on three human malignant cell lines by inducing apoptotic cell death. *J Photochem Photobiol B* 1998;43(2):106–11.
- [7] Gederaas OA, Lagerberg JW, Brekke O, Berg K, Dubbelman TM. 5-aminolevulinic acid induced lipid peroxidation after light exposure on human colon carcinoma cells and effects of alpha-tocopherol treatment. *Cancer Lett* 2000;159(1):23–32.
- [8] Kelley EE, Buettner GR, Burns CP. Production of lipid-derived free radicals in L1210 murine leukemia cells as an early oxidative event in the photodynamic action of Photofrin. *Photochem Photobiol* 1997;65(3):576–80.
- [9] Melnikova V, Bezdetnaya L, Belitchenko I, Potapenko A, Merlin JL, Guillemin F. Meta-tetra (hydroxyphenyl)-chlorine-sensitized photodynamic damage of cultured tumor and normal cells in the presence of high concentrations of alpha-tocopherol. *Cancer Lett* 1999;139(1):89–95.
- [10] Woods JA, Traynor NJ, Brancaleon L, Moseley H. The effect of photofrin on DNA strand breaks and base oxidation in HaCaT keratinocytes: a comet assay study. *Photochem Photobiol* 2004;79(1):105–13.
- [11] Allison RR, Cuenca R, Downie GH, Randall ME, Bagnato VS, Sibata CH. PD/PDT for gynecological disease: a clinical review. *Photodiagn Photodyn Ther* 2005;2(1):51–63.
- [12] Chekulayeva LV, Shevchuk IN, Chekulayev VA. Influence of temperature on the efficiency of photodestruction of Ehrlich ascites carcinoma cells sensitized by hematoporphyrin derivative. *Exp Oncol* 2004;26(2):125–39.
- [13] Schneider C. Chemistry and biology of vitamin E. *Mol Nutr Food Res* 2005;49(1):7–30.
- [14] Ahn WS, Bae SM, Huh SW, Lee JM, Namkoong SE, Han SJ, et al. Necrosis-like death with plasma membrane damage against cervical cancer cells by photodynamic therapy. *Int J Gynecol Cancer* 2004;14(3):475–82.
- [15] Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics 2006. *CA Cancer J Clin* 2006;56(2):106–30.
- [16] Gupta S, Dwarakanath BS, Muralidhar K, Jain V. Cellular uptake, localization and photodynamic effects of haematoporphyrin derivative in human glioma and squamous carcinoma cell lines. *J Photochem Photobiol B* 2003;69(2):107–20.
- [17] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico Biol Interact* 2006;160(1):1–40.
- [18] Harrison DJ. Molecular mechanisms of drug resistance in tumours. *J Pathol* 1995;175(1):7–12.
- [19] Kline K, Yu W, Sanders BG. Vitamin E: mechanisms of action as tumor cell growth inhibitors. *J Nutr* 2001;131(1):161S–3S.
- [20] Neuzil J, Weber T, Schröder A, Lu M, Ostermann G, Gellert N, et al. Induction of cancer cell apoptosis by alpha-tocopherol succinate: molecular pathways and structural requirements. *FASEB J* 2001;15(2):403–15.
- [21] Boscoboinik D, Szcwzyk A, Hensey C, Azzi A. Inhibition of cell proliferation by alpha-tocopherol. Role of protein kinase C. *J Biol Chem* 1991;266(10):6188–94.
- [22] Kline K, Yu W, Sanders BG. Vitamin E and breast cancer. *J Nutr* 2004;134(12 Suppl):3458S–62S.
- [23] Kumar KS, Raghavan M, Hieber K, Ege C, Mog S, Parra N, et al. Preferential radiation sensitization of prostate cancer in nude mice by nutraceutical antioxidant gamma-tocotrienol. *Life Sci* 2006;78(18):2099–104.
- [24] Cao EH, Zhang J, Li J. Initiation of apoptosis by photosensitization with hypocrellin A and the repression of vitamin E. *Proc SPIE* 1998;3548:38–44.
- [25] Liu S, Semenciw R, Mao Y. Cervical cancer: the increasing incidence of adenocarcinoma and adenosquamous carcinoma in younger women. *CMAJ* 2001;164(8):1151–2.