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Photodynamic therapy of tumours with chlorin-e_6 is pH dependent

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Abstract. Ehrlich ascites carcinoma (EAC) cells were used to investigate the effect of pH and glucose on the antitumour efficiency of photodynamic therapy (PDT) with chlorin-e_6 trimethyl ester (E6). For *in vitro* experiments, the cells were pre-incubated with E6 in a serum-free medium at pH 7.2 or 6.2 and were then irradiated with red light at 665 nm. It was found that their photosensitivity increased with decreasing pH value, although the cellular uptake of the sensitizer was slightly (by about 25%) depressed. As the pH value in malignant tumours tends to be lower than that of normal tissue and can be suppressed selectively by glucose administration, experiments were performed on mice bearing EAC cells grafted subcutaneously. An apparent delay in the tumour growth was obtained by combining E6–PDT with a single injection of glucose (2 g/kg body weight). This administration of glucose (2 h before the PDT) caused a substantial decrease in the tumour pH from an initial value of ~7.2 to ~6.3, as measured by a needle pH electrode. Studies on the mechanism suggest that under acidification the impairment of the mitochondrial function and the inhibition of E6 photobleaching in the cells are probably the main factors as to the revealed effect of pH on the antitumour efficiency of PDT with the sensitizer. It was also demonstrated that during E6–PDT along with singlet molecular oxygen hydroxyl free radicals are involved in the cell death.

Key words: chlorin-e_6, glucose, photobleaching, succinate dehydrogenase, superoxide, hydroxyl radical.

Abbreviations: ATP = adenosine triphosphate; DMSO = dimethyl sulphoxide; DEF = deferoxamine mesylate; EAC = Ehrlich ascites carcinoma; E6 = chlorin-e_6 trimethyl ester; GSH = reduced glutathione; HE = hydroethidine; H_2O_2 = hydrogen peroxide; i.p. = intraperitoneal; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $^1O_2$ = singlet oxygen; $O_2^{-\bullet}$ = superoxide anion; $OH^\bullet$ = hydroxyl radical; PBS = phosphate buffered saline; PDT = photodynamic therapy; PS = photosensitizer; ROS = reactive oxygen species; s.c. = subcutaneously; TB = trypan blue.

INTRODUCTION

The development of laser and fibre-optical techniques has provided new possibilities for the treatment of cancer. At present, low-power lasers are used in

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photodynamic therapy (PDT) of malignant tumours. PDT is based on the administration of a tumour-localizing drug (photosensitizer, PS) and its subsequent activation by laser light. Upon activation, PSs generate reactive oxygen species (singlet oxygen, $^1O_2$, and different free radicals), which are able to damage plasma membranes and other cellular structures, meaning that PDT can be a particularly useful alternative treatment for drug resistant tumours (Canti et al., 1995). Normal cells, however, are also able to accumulate PSs, so that prolonged skin photosensitivity as well as other side effects are some of the limitations of PDT.

Chlorin-e_6 and some of its derivatives are proposed as second-generation sensitizers for PDT of cancer. Several promising clinical studies with these PSs have been published (Taber et al., 1998). The main advantage of chlorin-e_6 like PSs in comparison with Photofrin (the most widely used drug for the PDT) is their strong absorption of light in the wavelength region around 660 nm. The mechanism of the chlorin-e_6 based PDT has been studied insufficiently, but it may involve a direct tumouricidal effect (damage to organelles within malignant cells) and also microcirculatory changes resulting in reduced blood flow (Chekulayev et al., 1991; Saito et al., 2000).

A number of procedures have been proposed to enhance the selectivity and efficiency of PDT. For instance, the use of PS conjugates with monoclonal antibodies against specific tumour cell receptors (Oseroff et al., 1986) and combinations of PDT with local hyperthermia (Henderson et al., 1985), ionizing radiation (Kostron et al., 1986), radiosensitizers of the hypoxic cells (Chekulayev et al., 1992), or some chemotherapeutic drugs (Cowled et al., 1987; Chekulayev et al., 1991) have been reported to lead to improved tumour and tumour cell response.

It is well known that the pH value in rapidly growing malignant tumours tends to be somewhat lower (due to an inadequate vascularization) than that of the surrounding normal tissue. The normal tissue pH in man varies from 7.0 to 8.06, whereas the pH values obtained in human tumours vary from 5.85 to 7.68 (Pottier & Kennedy, 1990). This has been proposed to be one of the reasons for the tumour selective uptake of several PSs (Pottier & Kennedy, 1990). Tumour pH can be driven selectively even lower by the administration of glucose in high concentrations (Thistlethwaite et al., 1987; Leeper et al., 1998). Numerous attempts have been made at exploiting this phenomenon to enhance the selective delivery and cytotoxicity of antineoplastic drugs (Kozin et al., 2001). However, the possibility of using this approach to improve the efficiency of PDT with chlorin-e_6-type PSs has not been explored. A low pH value might make tumour cells more vulnerable, for instance, by increasing their susceptibility to free radical toxicity due to an inhibition of the cellular repair enzymes activity. An additional tumour-selective effect might be expected for PSs of the porphyrin type, whose lipophilicity and cell uptake increase with decreasing pH (Thomas & Girotti, 1989a; Pottier & Kennedy, 1990).

In the present work we investigated, therefore, the effect of pH and glucose administration on the efficiency of PDT with chlorin-e_6 trimethyl ester (E6) using both *in vitro* and *in vivo* protocols.

MATERIALS AND METHODS

Chemicals

E6 was synthesized on the basis of pheophytin-*a* extracted from nettle leaves according to the procedure of Lötjönen & Hynninen (1980). The obtained product was more than 99.5% pure. Working solutions of E6 were dissolved in dimethyl sulphoxide (DMSO) at concentrations from 1 to 10 mM and were used on the same day as prepared. Other chemicals (of analytical grade or better) were purchased from Sigma, St. Louis, USA, unless noted otherwise.

Animals and tumours

White, mongrel, three-month-old female mice obtained from the Institute of Experimental and Clinical Medicine (Tallinn, Estonia) were used in the experiments. The animals were fed *ad libitum* on standard pellets and had permanent access to water. Ehrlich ascites carcinoma (EAC) cells obtained from the Institute of Chemical Physics and Biophysics (Tallinn, Estonia) were maintained by intraperitoneal (i.p.) transplantation of 0.25 mL ascites fluid (about 3×10^7 cells) from mouse to mouse every 7 days.

Preparation of cell suspensions and the irradiation conditions

For *in vitro* experiments, six- to seven-day-old EAC cells were withdrawn with a syringe from the abdominal cavities of the mice and a five-fold amount of cooled phosphate buffered saline (PBS) containing 154 mM NaCl, 6.2 mM KCl, 5.55 mM glucose, and 10 mM sodium phosphate buffer (pH 7.2) was added to the cells. The cell suspension was then stirred and centrifuged for 7 min at 4 °C. The packed cells were resuspended in the PBS to get a concentration of 1.2×10^8 cells/mL and kept in an ice bath until use. The viability of the cells was about 95–98%, as found by phase-contrast microscopy in the presence of trypan blue (TB). The washed cells were then diluted with PBS at pH 6.2 or 7.2 to a final concentration of 5×10^6 cells/mL. An 8 mL sample of the cell suspension was transferred into a 2×2 cm thermostatted quartz cuvette and a microstirring magnet was added. Further, the cells were incubated with 2 μM E6 in PBS at pH 6.2 or 7.2 for 25 min at 37 °C and were then irradiated at the same temperature under magnetic stirring. In the control experiments (irradiation of EAC cells in the absence of E6), the cells received the same amount of DMSO.

In all the experiments a voltage regulated 1 kW xenon arc lamp equipped with a parabolic mirror and glass filters (KS-14 together with SZS-25) to deliver the light at 665 nm (the range between 630 and 830 nm) served as the radiation source. The infrared radiation was removed by a 4-cm water filter. The flux of light was

focused as a spot (2.54 cm^2) and directed on the front face of a quartz cuvette containing EAC cell suspension. The intensity of the emitted light at 665 nm (for *in vitro* experiments) was 60 mW with a power density of 23.6 mW/cm^2, as measured by an IMO-2N radiometer (Russian Federation).

Cellular uptake of E6

To evaluate the cell uptake capacity of the drug, EAC cells (5×10^6 cells/mL) were incubated as described above with 2 μM E6 in PBS at pH 6.2 or 7.2 for 25 min at 37 °C. The pH value (6.2–7.2) of the PBS was adjusted by the addition of HCl or NaOH. After the 25 min incubation with E6, the cells were washed twice (by centrifugation) with ice-cold PBS (pH 7.2) to remove the unbound fraction of the drug. Finally the washed cells were resuspended in PBS (pH 7.2) to get a concentration of 5×10^6 cells/mL. The cellular fluorescence of E6 was measured at 672 nm using 1 cm × 1 cm quartz cuvettes. All fluorescence measurements were performed with a Hitachi 650-60 fluorescence spectrophotometer (Japan).

Assessment of cellular injury

Cytotoxicity was determined by the TB exclusion assay. After the treatments, an aliquot of the cell suspension (5×10^6 cells/mL) was diluted 1 : 1 (v/v) with 0.2% TB and 2 min later the cells were counted using a Goryaev's chamber. The results are expressed as the percentage of dead cells calculated as the ratio of TB-stained versus the total number of cells.

Assessment of the mitochondrial function

The effect of pH on cellular energy metabolism was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This assay is based on the reduction of MTT by the succinate dehydrogenase of the mitochondria (Mossman, 1983). For the determination of the dehydrogenase activity of EAC cells, the cells were resuspended in PBS at either pH 6.2 or 7.2 to a density of 5×10^6 cells/mL. Further the aliquots of cell suspension containing 5×10^5 cells were withdrawn in duplicate and were placed into test tubes. After the addition of 0.5 mL of a 2 mg/mL MTT solution (in PBS at pH 6.2 or 7.2), the cells were incubated for 3 h in a water bath at 37 °C. At the end of the incubation, the tubes with samples were immersed in an ice bath for 5 min, and the cells were harvested by centrifugation at $3000 \times g$ for 10 min at 4 °C. The supernatant was removed by aspiration and the pellet was dissolved in 2.5 mL DMSO. Absorbance of the samples was measured at 570 nm.

Intracellular content of reduced glutathione

Reduced glutathione (GSH) was assayed by the spectrofluorometric method of Hissin & Hilf (1976). Briefly, GSH in the acid-soluble supernatant fraction of EAC cells was reacted with *o*-phthaldialdehyde (Fluka BioChemica, Germany) at pH 8 to yield a highly fluorescent cyclic product.

Determination of superoxide anion

The formation of superoxide anion ($O_2^{-\bullet}$) in EAC cells subjected to E6–PDT was measured fluorometrically by the hydroethidine (HE) method essentially as described in (Rothe & Valet, 1990). HE crosses cell membranes and is oxidised by $O_2^{-\bullet}$ to ethidium bromide, which emits red fluorescence. A stock solution of HE (10 mM) was prepared in N,N-dimethylformamide and stored at −20 °C in aliquots of 10 μL. A 1 mM HE solution was prepared by adding 90 μL of PBS (pH 7.2) immediately before using. Following photoirradiation, aliquots of cell suspension (containing 2.25×10^6 cells) were incubated with 10 μM HE (in PBS, pH 7.2) for 15 min in a shaking water bath at 37 °C in 1.5 mL final volume. After the incubation, the fluorescence from ethidium bromide in the cells was measured at 610 nm (λ_{ex} = 488 nm) in 1 cm × 1 cm quartz cuvettes; slits were 5 and 15 nm for excitation and emission, respectively. The blank corresponding to HE alone in the buffer was subtracted from the value.

Tumour response to PDT with E6 combined with a single injection of glucose

The experiments were performed on 49 female mice with an average weight of 28 g to whom 0.2 mL EAC (2.4×10^7 cells) was inoculated subcutaneously (s.c.) on the back of mice anaesthetized briefly with ether. Tumour growth was documented twice per week by calliper measurements in three orthogonal diameters. Fourteen days after the inoculation, when the tumours reached 1–1.2 cm^3 (about 0.9 g), the mice were injected i.p. with E6 (10 mg/kg body weight) dissolved in DMSO. The PDT of tumour-bearing mice with E6 alone or combined with a single i.p. injection of glucose (2 g/kg, 2 h before photoirradiation) was carried out on the next day under narcosis (sodium thiopental, rectal injection) with red light at 665 nm. The irradiation apparatus was the same as for the *in vitro* experiments. The flux of light was focused as a spot (2.5 cm^2) and directed by means of an optical system into the region of tumour growth. The fluence rate at the position of the tumour was measured as 160 mW/cm^2. The light exposure time was 15 min corresponding to an exposure of 144 J/cm^2. For evaluation the mice were killed (by an i.p. injection of sodium thiopental) on the eighth day after their treatment and the weights of the tumours were determined.

The pH value of interstitial fluid in solid Ehrlich carcinomas was measured (under ether narcosis) on the fourteenth day after the tumour inoculation by a needle pH electrode (Diamond General Development Corp., USA), using the general procedure of Thistlethwaite et al. (1987).

RESULTS AND DISCUSSION

On the mechanism of cellular destruction under E6–PDT

Figure 1 shows that at E6-induced PDT the time course of EAC cells death was S-type. In the work, the light dose at which 50% of the cells were stained by TB (LD_{50}) was used as a criterion to evaluate the antitumour efficiency of E6–PDT *in vitro*. Control experiments showed that E6 itself was practically non-toxic towards EAC cells, since a prolonged incubation of the cells with the drug in the dark at 37 °C did not cause any noticeable decrease in the number of viable cells in the suspension (data not shown).

The tumouricidal effect of E6–PDT depends largely on the presence of oxygen (Chekulayev et al., 1992). However, until now the identity of the reactive oxygen species (ROS) mediating the PDT-induced tumour eradication has

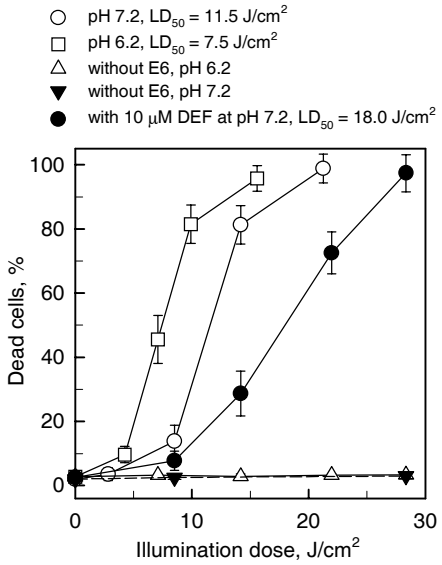

Fig. 1. Dose-response curves for photodynamic inactivation of EAC cells (5×10^6 cells/mL) at different pH values. The cells were incubated with 2 μM E6 in PBS at pH 6.2 or 7.2 for 25 min and were then irradiated without or in the presence of deferoxamine mesylate (DEF) at 37 °C. LD_{50} is the light dose at which 50% of the cells were stained by TB. All data are from three independent experiments. Bars designate standard errors (SE).

remained unclear. Studies performed in other laboratories (Bachor et al., 1991) indicated that $^1O_2$ plays an important role in the phototoxicity of chlorin-e_6 against malignant cells. However, our data suggest that under E6–PDT besides $^1O_2$, hydroxyl free radicals ($OH^\bullet$) are involved in the cell death. In fact, upon addition of deferoxamine mesylate (DEF), a well-known chelator of iron, the LD_{50} value of E6–PDT was increased by a factor of about 1.6 (Fig. 1); it was reported by Comelli et al. (1994) that in mammalian cells DEF can inhibit the reductive decomposition of hydrogen peroxide (H_2O_2) in the Fenton-type reactions preventing thereby the formation of very cytotoxic $OH^\bullet$. Moreover, using the HE assay we established (Fig. 2) that photoirradiation of E6-loaded cells leads to the production of significant amounts of $O_2^{-\bullet}$, a precursor of H_2O_2 and very reactive $OH^\bullet$. The generation of $O_2^{-\bullet}$ during E6–PDT could be explained in two ways. The first could be associated with oxidation of some biomolecules by $^1O_2$ (Buettner & Hall, 1987). The other way might involve direct electron transfer from photo-excited molecules E6 to molecular oxygen. In one of our previous studies we already demonstrated that E6, in comparison with other sensitizers proposed for the use in PDT of tumours, has an increased inclination to photoprocesses with charge transfer (Chekulayev et al., 1992).

Thus, the obtained results indicate that during E6-based PDT, besides $^1O_2$ other reactive oxygen intermediates are involved in cell killing.

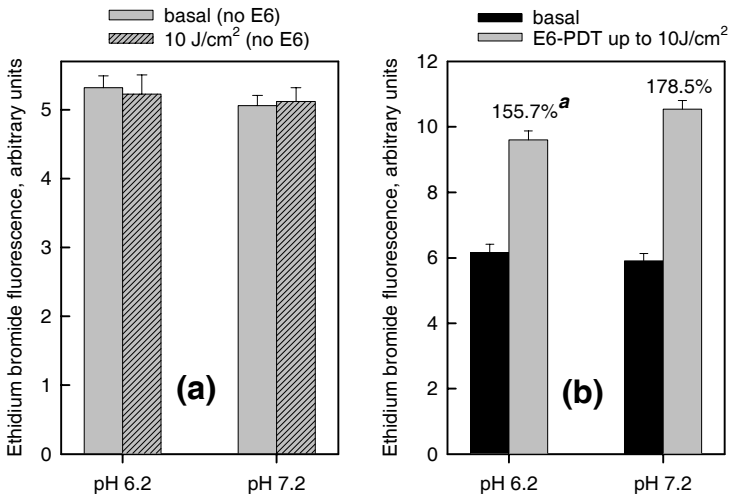

Fig. 2. Effect of pH on the intracellular formation of superoxide anions in EAC cells after their photoirradiation without (a) or in the presence of E6 (b). Before irradiation the cells (5×10^6 cells/mL) were incubated without or with 2 μM E6 in PBS at pH 6.2 or 7.2 for 25 min at 37 °C. The samples were read immediately following irradiation using the HE assay. All the data are from three independent experiments. Bars designate SE. [a] – % of basal.

Effects of pH on E6-based PDT *in vitro*

Figure 1 presents the dose-response curves for EAC cells subjected to E6–PDT 25 min after incubation with the photodrug in PBS at pH 7.2 or 6.2. These data show that at pH 6.2 the cell killing effect of PDT was significantly higher than at pH 7.2; the acidification caused a more than 1.5-fold increase in the inactivation rate of EAC cells, as found by measuring the LD_{50} value of PDT. It is important to note that at an extracellular pH of 6.2, the photoirradiation of EAC cells not exposed to E6 had no effect on the number of viable cells.

Several ways can be envisioned in which a decrease in the pH value might enhance cellular photosensitivity *in vitro*. It could be assumed that the cellular uptake of the PS is more efficient at a low pH than at pH values found in normal tissues, although E6 has no side groups whose protonation might increase its lipophilicity. Surprisingly, lowering the PBS pH from 7.2 to 6.2 decreased the accumulation of E6 in EAC cells by about 25% (Fig. 3). The mechanism of the phenomenon remains unclear and will be a subject of future studies. Thus, we cannot explain the increased phototoxicity of E6 at a low pH value by binding the PS to the cells.

It was reported (Spikes & Bommer, 1993) that the derivatives of chlorin-e_6 proposed for PDT of tumours are progressively destroyed (photobleached) during illumination and the process may potentially result in a reduced yield of inactivation of tumour cells per incident photon (Moan, 1986). Hence, another explanation for the pH dependence of E6-based PDT might be that photobleaching of the sensitizer is less efficient at acidic pH than at pH values close to neutral. To check the assumption, we evaluated the effect of pH on the photostability of E6 in tumour cells. Figure 4 indicates that upon illumination of EAC

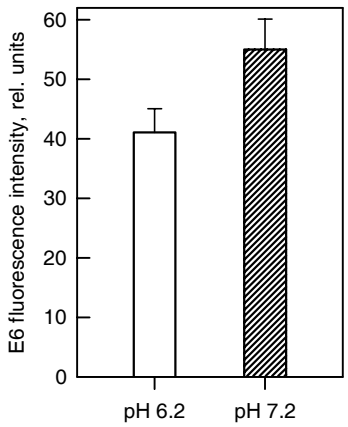

Fig. 3. Cellular uptake of E6 as measured by the cellular fluorescence intensity at 672 nm (λ_{ex} = 400 nm) as a function of pH. Before the measurements, EAC cells (5×10^6 cells/mL) were incubated with 2 μM E6 in PBS at pH 6.2 or 7.2 for 25 min at 37 °C. All the data are from three independent experiments. Bars designate SE.

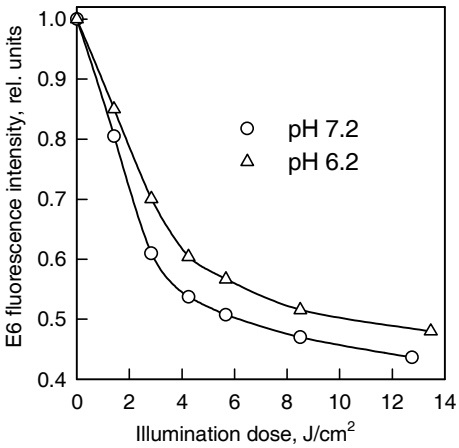

Fig. 4. Photoinduced degradation of E6 (as estimated by measuring the cellular fluorescence intensity of the dye at 672 nm, λ_{ex} = 400 nm) in EAC cells (5×10^6 cells/mL) at different pH values. The cells were incubated with 2 μM E6 in PBS at pH 6.2 or 7.2 for 25 min at 37 °C and were then irradiated at the same temperature under magnetic stirring (sample light path, 2 cm; fluence rate, 23.6 mW/cm^2). Each data point is the mean of three separate experiments. SE < 5%.

cells the drug undergoes a very rapid oxidation; however, lowering the pH value of PBS from 7.2 to 6.2 caused a substantial (1.44-fold) decrease in the initial rate of E6 photodestruction in the cells. These results could partly explain the elevated phototoxicity of E6 against the cells at a low pH value.

To achieve a more precise understanding of the influence of pH on the anti-tumour efficiency of E6–PDT, we measured the intracellular content of $O_2^{-\bullet}$ and GSH. The latter was chosen as a biomarker of oxidative stress because the peptide may react directly with some ROS, such as $^1O_2$ and $OH^\bullet$ (Rougee et al., 1988). Moreover, GSH and certain GSH-dependent enzymes play an important role in the protection of photodynamically treated cells against H_2O_2 as well as in the decomposition of lipid peroxides (Thomas & Girotti, 1989b). It was established (Fig. 2) that the photoirradiation of E6-labelled EAC cells at a low pH value (6.2) did not mediate a more efficient formation of $O_2^{-\bullet}$, a known precursor of very reactive $OH^\bullet$, which is found (Fig. 1) to be involved in the phototoxic action of the sensitizer. In addition, further studies revealed that the EAC cells subjected to E6–PDT at pH 6.2 had higher levels of GSH in comparison with those treated at pH 7.2 (Fig. 5). Thus, it seems to be unlikely that the increased phototoxicity of E6 toward the cells at acidic pH is mediated by a more pronounced oxidative stress.

At the same time, we found that the acidification of EAC cells causes a serious inhibition of their mitochondrial activity. Indeed, lowering the PBS pH from 7.2 to 6.2 led to a strong (almost 3-fold) decrease in the dehydrogenase activity of the cells in the MTT-assay (Fig. 6). Our results on the effect of pH on the energy

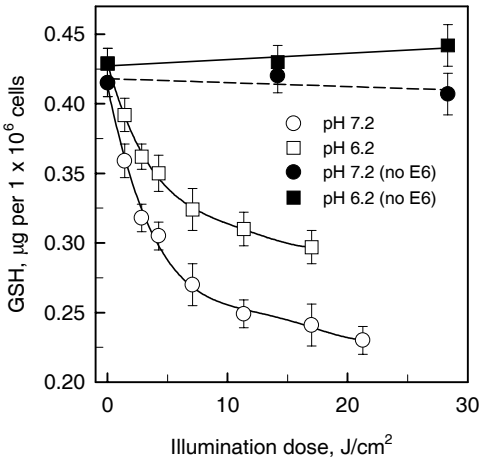

Fig. 5. The content of reduced glutathione (GSH) in EAC cells subjected to E6-based PDT at different pH levels. Experimental conditions were the same as in Figs. 1–4. The points with error bars refer to three independent experiments.

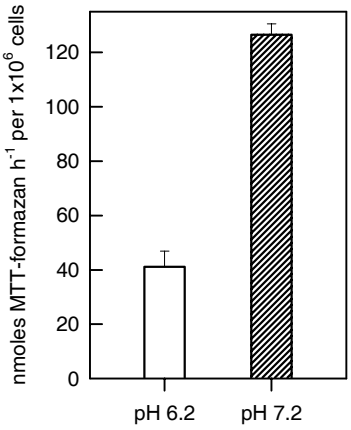

Fig. 6. Dehydrogenase activity of EAC cells in the MTT-assay as a function of pH. All the data are from three independent experiments. Bars designate SE.

metabolism of tumour cells are in good agreement with the data from other laboratories. For instance, Gabai & Mosin (1991) showed that the oxygen consumption of EAC cells is strongly (by 4-fold) inhibited after a decrease in pH from 7.3 to 6.0. In addition, these authors reported that at pH 6.0 the cells have a low level of glycolysis. Similar results were also obtained on the tumour cells of another histological type (Casciari et al., 1992). On this basis, we suppose that these acidic pH-induced disturbances of energy metabolism must cause a

substantial decrease in the adenosine triphosphate (ATP) content of neoplastic cells and thereby increase their sensitivity to E6–PDT. This assumption is very probable, because in our previous studies (Chekulayev et al., 1991) we established that suppression of mitochondrial oxidative phosphorylation, which coincided in time with a drastic decrease in the ATP level, is a crucial event in the cytotoxicity resulting from E6-induced photosensitization *in vitro*.

Influence of glucose administration on the efficiency of E6-based PDT

Numerous studies have demonstrated that induction of experimental hyperglycaemia in tumour-bearing animals (Thomas et al., 1989a; Volk et al., 1993) and humans (Thistlethwaite et al., 1987; Leeper et al., 1998) results in a significant lowering of the tumour pH. In an attempt at exploiting this phenomenon to enhance the antitumour efficiency of E6–PDT, we, first of all, evaluated the effect of glucose administration on the pH of the interstitial fluid in Ehrlich carcinomas. As shown in Table 1, a single i.p. injection of glucose (2 g/kg) resulted in a substantial decrease in the pH from an initial value of ~7.2 to ~6.3 after 2 h. Similar effects of glucose administration on the pH in experimental tumours were also observed in other laboratories (Thomas et al., 1989a; Volk et al., 1993). It is important to note that the glucose-induced tumour pH drop can persist for 24 h (Osinsky et al., 1987). In this work, the mechanism of the effect of glucose dose on pH depression was not explored; however, previous studies (Hiraoka & Hahn, 1990) clearly indicate that increased acidity is a consequence of elevated lactic acid production, although reduced blood flow in the tumour vasculature could be a contributing factor.

Further we explored the effect of glucose administration on the antitumour efficiency of E6–PDT of mice bearing s.c. transplanted EAC cells. Since the cellular uptake of the PS was slightly depressed in acidic conditions (Fig. 3), in the *in vivo* experiments the injection of glucose (up to 2 g/kg) to the tumour-bearing animals was carried out on the next day after the administration of E6 and

Table 1. Effect of glucose administration on the pH in s.c. transplanted Ehrlich carcinomas

Treatment[a]	Tumour pH, mean ± SE	Number of mice	*P*-value
Control	7.18 ± 0.05	7	–
Glucose	6.32 ± 0.06	7	< 0.01

[a] The tumour-bearing mice received a single i.p. dose of glucose (2 g/kg) or an i.p. injection of 0.9% NaCl (control animals); the pH in the tumours was measured 2 h after corresponding injections; the statistical significance of data obtained on control animals versus those on the glucose-treated mice was determined with Student's *t*-test.

Table 2. Influence of glucose administration on the efficiency of E6–PDT of mice bearing s.c. transplanted solid Ehrlich carcinomas

Treatment modality	Weight of tumours, g ± SE	Number of animals	*P*-value
Control	1.79 ± 0.12	10	–
PDT only[a]	1.11 ± 0.05 (62.0%[b])	8	<0.01
PDT 2 h after an i.p. injection of glucose (2 g/kg)	0.67 ± 0.07 (37.4%)	9	<0.01
Glucose only	1.76 ± 0.09 (98.3%)	8	–

Notes: The statistical significance of control animals versus the PDT-treated mice was determined with Student's *t*-test;
[a] the tumour-bearing mice received, instead of glucose, an i.p. injection of 0.9% NaCl;
[b] % of control.

2 h before light exposure. An apparent delay in the tumour growth was found by combining PDT with a single dose of glucose (Table 2). It is important to emphasize that the same injection of glucose, but without E6–PDT, had no effect on the rate of the tumour growth. As the PDT-induced death of EAC cells is sensitive to the pH within the range 6.2–7.2 (Fig. 1), the data presented in Table 1 suggest that the potentiating effect of glucose on the antitumour efficiency of E6-PDT *in vivo* could be ascribed to a decrease in the pH value of the tumours.

It is important to note that tumour cells are sensitive to hyperthermia and that glucose can potentiate this effect (van den Berg et al., 2001). Hyperthermia has been considered to be a possible contributory factor in tumour eradication by PDT (Kinsey et al., 1983). However, under the relatively mild irradiation conditions used in this study, it is unlikely that local heating played any significant role (Henderson et al., 1984).

CONCLUSIONS

In the present work we have demonstrated that tumour cells in an acidic milieu (both *in vitro* and *in vivo*) are more sensitive to E6-induced photosensitization than at pH values found in normal tissues. Studies on the mechanism suggest that under acidification the impairment of the mitochondrial function as well as the inhibition of E6 photobleaching in the cells are probably the main factors as to the revealed effect of pH on the antitumour efficiency of PDT with the sensitizer. Our findings suggest a new strategy for improving the selectivity and the cure rate of PDT with chlorin-e_6-type sensitizers, i.e. the induction of transient hyperglycaemia, which causes a significant drop in the pH value of the interstitial fluid of malignant tumours.

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Kasvajate fotodünaamiline teraapia kloriin-e_6 manulusel on pH-st sõltuv

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Ehrlichi astsiitse kartsinoomi (EAK) rakkudel uuriti pH ja glükoosi mõju kloriin-e_6 trimetüülestri (E6) manulusel fotodünaamilise teraapia (FDT) kasvajavastasele toimele. *In vitro* katsetes pH 7,2 või 6,2 juures inkubeeriti rakke E6-ga

ning seejärel kiiritati valguskiirgusega 665 nm. Rakkude fototundlikkus suurenes pH alanemisega, kuigi sensibilisaatori seostuvus rakkudega vähenes veidi (~25%). Pahaloomulistel kasvajatel on kalduvus madalamale pH-le võrreldes normaalsete kudedega ja pH-d saab selektiivselt alandada manustades glükoosi. Katsetes kasutasime hiiri, kellele oli EAK nahaaluselt poogitud. Ilmne kasvaja kasvu aeglustumine esines, kui E6–FDT kombineeriti ühekordse glükoosi injektsiooniga (2 g/kg kaalu kohta). Selline glükoosi manustamine (2 tundi enne FDT-d) põhjustas kasvaja pH olulise alanemise algväärtuselt 7,2 kuni ~6,3-ni, mida mõõdeti pH nõelelektroodiga. Selle nähtuse toimemehhanismi uurimine näitab, et pH alanemisel on arvatavasti mitokondrite vigastamine ja E6 fotolagunemise inhibeerimine rakkudes põhilisteks faktoriteks, mis tingivad pH mõju E6–FDT kasvajavastasele toimele. Leiti, et lisaks singletsele hapnikule on rakkude E6–FDT hävimisel oluline osa hüdroksüülradikaalidel.