Proc. Estonian Acad. Sci. Chem., 2000, **49**, 2, 109–120
https://doi.org/10.3176/chem.2000.2.03

L-BUTHIONINE-[S,R]-SULFOXIMINE MAY ENHANCE THE RATE OF HAEMATOPORPHYRIN DERIVATIVE PHOTOSENSITIZED INACTIVATION OF TUMOUR CELLS WITHOUT LOWERING THE INTRACELLULAR CONTENT OF GLUTATHIONE

Lyudmila CHEKULAYEVA, Igor SHEVCHUK*, Raissa JÄÄLAID, and Vladimir CHEKULAYEV

Institute of Chemistry, Tallinn Technical University, Akadeemia tee 15, 12618 Tallinn, Estonia; igor@chemnet.ee

Received 7 October 1999

Abstract. Haematoporphyrin derivative (HpD) is the most widely used photosensitizer (PS) for photodynamic therapy (PDT) of cancer. In the present study it was found that L-buthionine-[S,R]-sulfoximine (L-BSO), an inhibitor of glutathione (GSH) biosynthesis, added (jointly with HpD) to Ehrlich ascites carcinoma (EAC) cells for 5 min prior to photoirradiation (λ_{max} = 630 nm, the range 590–830 nm) enhanced synergistically the rate of HpD-sensitized photoinactivation of the cells. Thus, L-BSO, at a concentration of 2.5 mM caused (as measured by trypan blue test and MTT assay) a substantial (approximately 20%) increase in the HpD-induced photocytotoxicity. However, this effect of potentiation was not observed when chlorin-e_6 trimethyl ester was used as a PS. Studies on the mechanism revealed that the potentiating action of L-BSO on HpD–PDT was not induced by lowering the reduced GSH (a natural antioxidant) content in EAC cells. At the same time, L-BSO was found to weakly but significantly augment the uptake of HpD by the cells at all concentrations used (from 0.5 up to 2.5 mM). This finding explains the potentiating effect of L-BSO on the HpD-induced photokilling of EAC cells.

Key words: photodynamic therapy, haematoporphyrin derivative, tumour cells, buthionine sulfoximine.

Abbreviations: L-BSO = L-buthionine-[S,R]-sulfoximine; DMSO = dimethyl sulphoxide; EAC = Ehrlich ascites carcinoma; E6 = chlorin-e_6 trimethyl ester; GSH = glutathione; HpD = haematoporphyrin derivative; HBSS = Hanks'-balanced salt solution, without phenol red, supplemented with 10 mM Hepes buffer, pH 7.4; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $^1O_2$ = singlet oxygen; PS = photosensitizer; PDT = photodynamic therapy; TB = trypan blue.

* To whom correspondence and reprint requests should by addressed.

INTRODUCTION

Haematoporphyrin derivative (HpD) is a porphyrin mixture used as an anticancer agent in photodynamic therapy (PDT) [1]. The clinical treatment regime typically involves the intravenous administration of HpD followed by an equilibration period of 24–72 h, during which the "biologically active" porphyrin components of this photosensitizer (PS) are retained in tumour loci at greater concentrations than in normal tissues. Subsequently the malignancies are exposed to visible radiation, usually 630 nm laser light. Photoexcitation of the tumour-localizing components of HpD leads to the formation of singlet molecular oxygen ($^1O_2$), a highly reactive oxidant, which is reported to be the main agent responsible for induction of tumour necrosis [2]. Being hydrophobic in character, HpD tends to localize in plasma and subcellular membranes, making these structures especially sensitive to the oxidative photodamage [3–6]. $^1O_2$, formed upon illumination of HpD, may initiate the oxidative destruction of unsaturated phospholipids and cholesterol in cell membranes [7, 8]. Therefore, some researchers [9, 10] speculated that lipid peroxidation may play an important role in the cytolethal effects of PDT with HpD.

Glutathione (GSH) and some GSH-dependent enzymes have, as known [7], the key role in the protection of cells against toxic action of H_2O_2 and also in the decomposition of lipoperoxide compounds. In this connection, it has been shown both *in vitro* [9, 11] and *in vivo* [12] that GSH depletion in tumour cells by buthionine-[S,R]-sulfoximine (BSO), a selective inhibitor of GSH biosynthesis [13], increases substantially their sensibility to the lethal action of HpD–PDT. These data suggest that BSO might be useful for improvement of clinical PDT.

It is well known that porphyrins, including HpD components, bleach on illumination. The photodegradation of HpD may result in a reduced yield of inactivation of tumour cells per incident photon [14]. At the same time, the formation of photoproducts that have an absorption maximum around 640 nm (photoproduct-640) was also detected during photoirradiation of HpD not only in water solutions [15, 16], but also in tumour cells [17]. This may play a very important role in HpD–PDT of cancer because of an increased absorbance in the spectral region of higher tissue transmission. Furthermore, the formed photoproducts (which are probably reduced porphyrins) may have photodynamic activity [18]. Recently we found that upon illumination the addition of BSO to HpD solutions augments (by an unknown way) the yield of photoproduct-640 [15]. Hence, it could be assumed that BSO is able to enhance the rate of HpD-photosensitized inactivation of tumour cells by another way, particularly, via the stimulation of the above-mentioned photoproduct(s) formation. In the present work we performed, therefore, a more detailed investigation of the effects of BSO on phototoxicity of HpD towards tumour cells.

MATERIALS AND METHODS

Chemicals

HpD was prepared from haematoporphyrin IX dihydrochloride (Aldrich) according to the original method of Lipson et al. [19] modified by Kessel at al. [20]. The obtained product was diluted with 0.9% NaCl solution (pH 7.4) to a final porphyrin concentration of 5 mg/mL, and stored in the dark at –70°C. The working solution of HpD, at a concentration of 0.5 mg/mL, was prepared immediately before use by further diluting the stock solution with a medium (see below). Chlorin-e_6 trimethyl ester (E6) was synthesized on the basis of pheophytin-a extracted from nettle leaves according to the procedure of Lötjönen and Hynninen [21]. Working solutions of E6 were dissolved in dimethyl sulphoxide (DMSO) at a concentration of 2 mM and were used on the same day as prepared. L-BSO, 2-amino-4-(S-butylsulphonimidoyl)butanoic acid, and all other chemicals were purchased from Sigma Chemical Co., St. Louis, USA.

Animals and cells

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 in the mice by weekly transplantation, intraperitoneally, 3×10^7 cells per animal.

Light source

In all the experiments a 1 kW xenon arc lamp, which has a continuous character of light emission from 200 nm up to the infrared (IR) domain of the solar spectrum, equipped with glass filters (KS-10 together with SZS-25) that transmit 50% of the light at 630 nm (the range between 590–830 nm), and a focusing optical system served as the radiation source. The IR radiation was removed by a 4 cm water filter. The flux of light was focused as a spot (D = 1.8 cm) and directed on the front face of a quartz cuvette containing EAC cell suspension. In this study, the intensity of the emitted light at 630 nm was always 220 mW with a power density of 86.6 mW/cm^2, as measured with an IMO-2N radiometer (Russian Federation). EAC cells were also irradiated with 665 nm light (when E6 was used as a PS), by means of the glass filters (KS-14 together with SZS-25; bandpass range 630–830 nm). The intensity of the emitted light at 665 nm was 200 mW with a power density of 78.7 mW/cm^2.

Preparation of cell suspensions and the irradiation conditions

For *in vitro* experiments 6–8 days old EAC cells were withdrawn with a syringe from the mouse abdominal cavity and a five-fold amount of cooled medium (Hanks'-balanced salt solution, without phenol red, supplemented with 10 mM Hepes buffer, pH 7.4 (HBSS)) was added to the cells. The cell

suspension was then stirred and centrifuged for 8 min at 4 °C. The packed cells were resuspended in the above-mentioned medium 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). Finally, the cells were diluted with HBSS to a concentration of 5×10^6 cells/mL. Then, 5 min after the addition of a PS, the cells were irradiated at 630 or 665 nm for HpD and E6, respectively. Our prior studies [17, 22] showed that after a short incubation time (5 min) approximately 20–25% of the initial content of HpD (1 μg/mL) or E6 (2 μM) was bound to EAC cells. It was also found that the presence in a medium of non-bound PSs has a negligible effect on the rate of HpD- or E6-sensitized photodamage of the cells. The irradiation was carried out in a quartz cuvette (with optical path length of 2 cm; volume, 8 mL) by magnetic stirring at 20 °C. A 20 mM solution of L-BSO in HBSS (prepared immediately before use) was administered to the EAC cell suspension for 5 min prior to photoirradiation.

Determination of the amount of injured cells and activity of dehydrogenases

The amount of injured cells in the suspension was determined immediately after irradiation by staining the cells with 0.1% TB in phosphate-buffered saline (pH 7.2) as described earlier in [22]. Additionally, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used. This assay is based on the ability of mitochondrial dehydrogenases (at the sites of cytochromes b and c) in viable cells to reduce the yellow form of MTT to a purple formazan [23]. For the determination of the dehydrogenase activity of EAC cells, the fractions of 0.1 mL (containing 5×10^5 cells) were withdrawn in duplicate and were placed into test tubes. The cells were then washed once in ice-cold HBSS. After the addition of 0.5 mL of 2 mg/mL MTT solution (in HBSS), EAC cells were incubated for 2.5 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 mL DMSO. Optical absorbance of the converted dye was measured at 570 nm. The molar extinction coefficient for MTT–formazan in DMSO, needed to calculate its content in the samples, was determined experimentally as 1.35×10^4 $M^{-1}cm^{-1}$.

Tryptophan content of EAC cells

In a previous study [24] we found that EAC cells have a clearly expressed fluorescence. At the excitation wavelength of 296 nm the maximum of their fluorescence lies in the 333 nm band. Our results [24] and the data of other laboratories [25], in turn, indicate that the tryptophanyl residues in proteins are mainly responsible for the cellular fluorescence at 330 nm. On this basis, the tryptophan content of EAC cells after their photoirradiation in the presence of HpD (with or without L-BSO) was evaluated fluorometrically. The light-induced fluorescence at 333 nm from EAC cells (5×10^6 cells/mL in HBSS) was

measured in a quartz cuvette with optical path length of 1 cm under magnetic stirring at 20°C. The fluorescence intensities were registered by a spectrofluorometer Hitachi 650-60 (Japan) with 2 nm band widths for excitation and detection.

Determination of the rate of photoproduct-640 formation

Since the photoproduct-640 has, as found in [15], the fluorescence maximum around 644 nm, the rate of its formation during illumination of HpD in EAC cell suspension (without or in the presence of L-BSO) was monitored fluorometrically. These measurements were performed in a quartz cuvette with an optical path length of 1 cm under magnetic stirring at 20°C. The kinetics experiments were repeated three times.

Intracellular content of reduced GSH

Reduced GSH in EAC cells was determined with 5,5'-dithiobis-2-nitrobenzoic acid exactly as described in one of our earlier studies [22].

Determination of cellular uptake of HpD

In a typical experiment, 8 mL of EAC cell suspension (5×10^6 cells/mL in HBSS) was placed into a quartz cuvette ($2 \times 2 \times 2.5$ cm). The cuvette was provided with magnetic stirring. After the administration of HpD, up to a final concentration of 2 μg/mL, the cell suspension was incubated (without or in the presence of L-BSO) in the dark at 20°C. The fractions of 0.5 mL cellular suspension were withdrawn in triplicate 30 min after the addition of HpD and were placed in 2.0 cm^3 plastic tubes (from Sigma). The cells were then sedimented by centrifugation at $3000 \times g$ for 5 min at 20°C. The supernatant was removed by aspiration. The total amount of HpD in the cells was measured by dissolving the cells in 1.5 mL of 1.0 M NaOH and by measuring the fluorescence (excitation at 394 nm) of this solution at 617 nm. The porphyrin concentrations were determined from a calibration curve, which was obtained by measuring the fluorescence intensity of known HpD concentrations in 1.0 M NaOH. The calibration curve was linear up to 0.5 μg HpD per mL. Therefore, the analysed samples were diluted below this value.

RESULTS AND DISCUSSION

Figure 1 shows that at HpD-induced PDT the time course of EAC cells photodamage was S-type. Indeed, after the lag period (24 min) during which the number of stained cells remained at the control level, a strong increase in the rate of uptake of TB was observed. This indicates an essential damage of plasma membranes in the cells. In addition, during the lag period (Fig. 1) a powerful (about 70%) inhibition of dehydrogenase activity of EAC cells took place (Fig. 2). The measurements of dehydrogenase activity by MTT assay demonstrate that HpD–PDT also caused an essential injury of the mitochondria

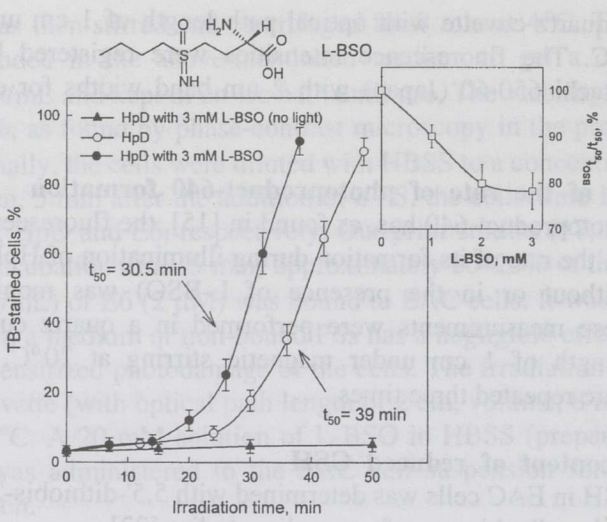

Fig. 1. The rate of trypan blue (TB) staining of EAC cells after 1 μg/mL HpD–PDT without or in the presence of L-BSO. Inset: Effect of L-BSO concentration on the efficiency of HpD-sensitized photodamage of EAC cells. t_{50}, irradiation time at which 50% of the cells were stained by TB ($^{BSO}t_{50}$, the same with L-BSO). Each data point is the mean of three separate experiments; bars = SE.

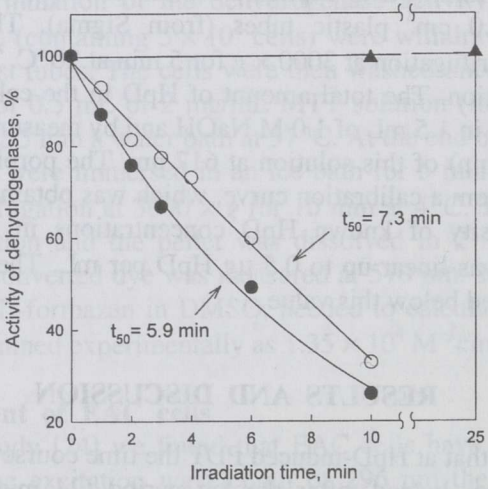

Fig. 2. Effects of HpD-induced photosensitization (HpD, 1 μg/mL) without or in the presence of L-BSO on the dehydrogenase activity of EAC cells. The initial activity of dehydrogenases of the non-irradiated cells was determined as 78.6 ± 1.6 nmoles MTT–formazan h^{-1} per 1×10^6 cells; the value was taken as 100%. t_{50}, irradiation time at which 50% decrease of dehydrogenase activity of the cells was observed. Each data point is the mean of three separate experiments performed in duplicate. SE < 5%.

of the cells. At the same time, photoirradiation of EAC in the absence of HpD, as well as their incubation with the PS in the dark, did not mediate any noticeable increase in the number of injured cells (data not shown). In the present study, the irradiation time at which 50% of cells were stained by TB (t_{50}) was used as a criterion to evaluate the efficiency of HpD-induced photocytotoxicity.

Besides the effects described above, HpD–PDT produced a noticeable decrease in the tryptophan content in EAC cells, as indicated by the measurements of cellular fluorescence in the 333 nm band (Fig. 3). Hence, the photodynamic treatment caused an effective damage to cellular proteins.

The study showed that HpD-mediated photosensitization caused a strong violation not only to the cytoplasmic membrane but also to the energy homeostasis of EAC cells. Our data are in a good agreement with those of other researchers [4] who believe that mitochondria are primary targets of HpD–PDT. Indeed, a considerable inhibition of cellular respiration (Fig. 2), which was associated with a dramatic (approximately 80%) fall in the intracellular content of adenosine triphosphate [26], preceded the photodestruction of the outer membrane in the cells (Fig. 1). Moreover, the results obtained suggest that HpD-photosensitized degradation of proteins, besides photoperoxidation of lipids, may be responsible for the increase in membrane permeability in EAC cells (Fig. 1). Thus, at a light dose that irreversibly inactivated about 90% of the cells (as measured by TB test) a clearly expressed (approximately 5%) diminution of the tryptophan content in cellular proteins was observed (Fig. 3).

Figure 1 depicts the influence of L-BSO on the efficiency of HpD-induced photocytotoxicity. These results demonstrate that L-BSO added to EAC cells (jointly with HpD) 5 min prior to light exposure enhanced synergistically the rate of HpD-sensitized photoinactivation of the cells within the whole range of the

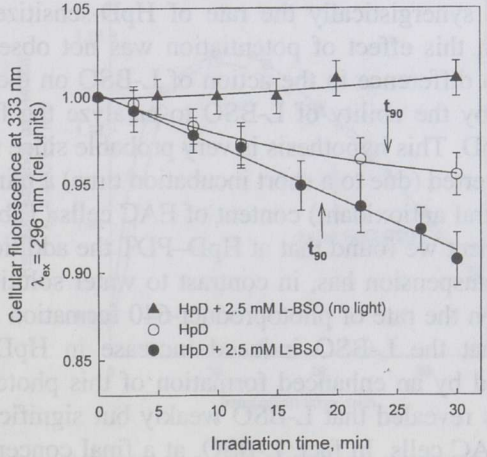

Fig. 3. The tryptophan content of EAC cells (5×10^6 cells/mL) after 1 μg/mL HpD–PDT without or in the presence of 2.5 mM L-BSO. t_{90}, irradiation time at which 90% of the cells were stained by TB; bars = SE.

concentrations used (from 1.0 up to 3 mM). Thus, the presence of 1 mM L-BSO caused (as measured by TB test) a small (approximately 10%) increase in HpD–PDT efficiency (the inset to Fig. 1). At a higher concentration of L-BSO (2 mM), the t_{50} exposure of PDT was already decreased by about 20%. However, increasing the L-BSO concentration in EAC cell suspension from 2 up to 3 mM did not provide any further growth in the cytotoxicity of HpD–PDT. Similar effects of L-BSO on the efficiency of HpD–PDT were also registered when ethidium bromide was used as a vital dye (data not included). In addition, it was found that L-BSO essentially enhanced the destructive impact of HpD–PDT on mitochondrial components of tumour cells. Indeed, at a concentration of 2.5 mM of L-BSO, the irradiation time required for 50% inhibition of the dehydrogenase activity of EAC cells was decreased by approximately 20% (Fig. 2). Moreover, the administration of L-BSO (up to 2.5 mM) enhanced the rate of HpD-photosensitized oxidation of cellular proteins, as indicated by the measurements of the tryptophan content in EAC cells (Fig. 3). It is important to emphasize that L-BSO itself, without PDT, was practically nontoxic towards EAC cells. In fact, a prolonged incubation of the cells with L-BSO (2.5–3.0 mM) did not mediate any noticeable increase in the number of TB-stained cells (Fig. 1) and caused only a negligible effect on dehydrogenase activity of EAC cells (Fig. 2); the same effects were observed when the cells were photoirradiated in the presence of L-BSO but without HpD (data not shown).

We investigated the influence of L-BSO on the intensity of photodamage of EAC cells also in the presence of E6. Thanks to its better spectral properties, E6 might be an alternative PS to HpD [26]. Moreover, E6, in contrast to HpD, is able to react in type 1 photoreactions [27]. The results obtained show (Fig. 4) that in the case of E6, L-BSO, at a concentration of 2 mM, had a negligible effect on PDT-induced cytotoxicity.

Thus, in the study we found that L-BSO added immediately before photoirradiation enhanced synergistically the rate of HpD-sensitized photodamage of EAC cells. However, this effect of potentiation was not observed when E6 was used as a PS. Such a difference in the action of L-BSO on the efficiency of PDT might be explained by the ability of L-BSO to catalyze the formation of photoproduct-640 from HpD. This hypothesis is very probable since in our experimental conditions L-BSO exerted (due to a short incubation time) a minor influence on the reduced GSH (a natural antioxidant) content of EAC cells (Table 1). Nevertheless, in a separate experiment we found that at HpD–PDT the addition of L-BSO (up to 3 mM) to EAC cell suspension has, in contrast to water solutions of the PS [15], only a minor effect on the rate of photoproduct-640 formation (Fig. 5). Therefore, it seems unlikely that the L-BSO induced increase in HpD photocytotoxicity (Fig. 1) was mediated by an enhanced formation of this photoproduct. However, further investigations revealed that L-BSO weakly but significantly augments the uptake of HpD by EAC cells. In fact, L-BSO, at a final concentration of 2.5 mM, caused a noticeable (approximately 15%) increase in the cellular HpD content (Fig. 6). These findings explain, in our opinion, the potentiating effect of L-BSO on the HpD-induced photokilling of EAC cells.

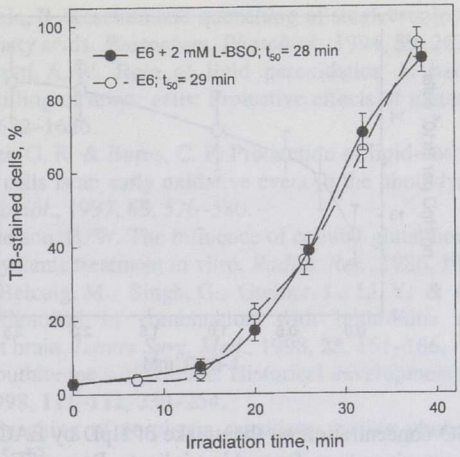

Fig. 4. The rate of trypan blue (TB) staining of EAC cells after 2 μM E6–PDT without or in the presence of 2 mM L-BSO. t_{50}, irradiation time at which 50% of the cells were stained by TB; bars = SE.

Table 1. The influence of L-BSO on the reduced GSH content in EAC cells*

Incubation time, min	Reduced GSH, nmoles per 1×10^6 cells ± SE	% of initial content
0	3.14 ± 0.10	100
30	3.1 ± 0.12	98.7
60	3.0 ± 0.10	95.5

* After the addition of L-BSO, up to a final concentration of 2.5 mM, the cells (4×10^7 cells/mL in HBSS) were incubated in a quartz cuvette (volume, 8 mL) under magnetic stirring at 20 °C.

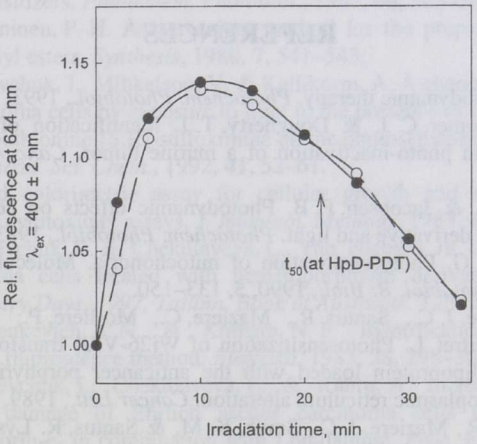

Fig. 5. The rate of photoproduct-640 formation during illumination (λ_{max} = 630 nm) of HpD (C_0 = 1 μg/mL) in EAC cell suspension (5×10^6 cells/mL in HBSS) without (○) or in the presence of 3 mM L-BSO (●). t_{50} is the irradiation time at which 50% of the cells were stained by TB. SE < 5%.

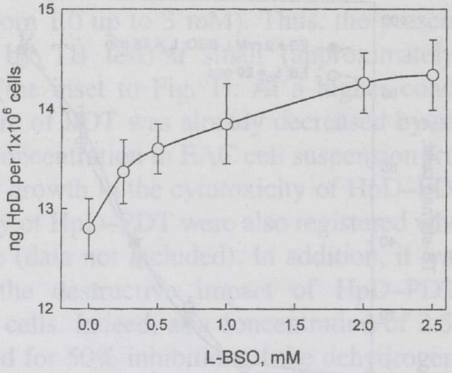

Fig. 6. The effect of L-BSO concentration on the uptake of HpD by EAC cells. Each data point is the mean of three separate experiments performed in triplicate. Bars = SE.

CONCLUSIONS

In the present study it is shown that L-BSO (a known inhibitor of GSH biosynthesis) at 1–2.5 mM, i.e. at concentrations that are clinically achievable and do not cause severe side effects [28], may increase synergistically the HpD-induced phototoxicity not only via GSH depletion (as found before by others [9, 11]) but also because of its ability to enhance the uptake of HpD in neoplastic cells. Hence, at clinical PDT the maximum value of tumour necrosis may, probably, be achieved by the joint administration of L-BSO with HpD.

REFERENCES

1. Dougherty, T. J. Photodynamic therapy. *Photochem. Photobiol.*, 1993, **58**, 895–900.
2. Weishaupt, K. R., Gomer, C. J. & Dougherty, T. J. Identification of singlet oxygen as the cytotoxic agent in photo-inactivation of a murine tumor. *Cancer Res.*, 1976, **36**, 2326–2329.
3. Moan, J., McGhie, J. & Jacobsen, P. B. Photodynamic effects on cells in vitro exposed to hematoporphyrin derivative and light. *Photochem. Photobiol.*, 1983, **37**, 599–604.
4. Salet, C. & Moreno, G. Photosensitization of mitochondria. Molecular and cellular aspects. *J. Photochem. Photobiol. B: Biol.*, 1990, **5**, 133–150.
5. Candide, C., Maziere, J. C., Santus, R., Maziere, C., Morliere, P., Reyftmann, J. P., Goldstein, S. & Dubertret, L. Photosensitization of Wi26-VA4 transformed human fibroblasts by low density lipoprotein loaded with the anticancer porphyrin mixture photofrin II: Evidence for endoplasmic reticulum alteration. *Cancer Lett.*, 1989, **44**, 157–161.
6. Geze, M., Morliere, P., Maziere, J. C., Smith, K. M. & Santus, R. Lysosomes, a key target of hydrophobic photosensitizers proposed for photochemotherapeutic applications. *J. Photochem. Photobiol. B: Biol.*, 1993, **20**, 23–35.
7. Thomas, J. P. & Girotti, A. W. Photooxidation of cell membranes in the presence of hematoporphyrin derivative: Reactivity of phospholipid and cholesterol hydroperoxides with glutathione peroxidase. *Biochim. Biophys. Acta*, 1988, **962**, 297–307.

8. Tanielian, C. & Mechin, R. Reaction and quenching of singlet molecular oxygen with esters of polyunsaturated fatty acids. *Photochem. Photobiol.*, 1994, **59**, 263–268.
9. Thomas, J. P. & Girotti, A. W. Role of lipid peroxidation in hematoporphyrin derivative-sensitized photokilling of tumor cells: Protective effects of glutathione peroxidase. *Cancer Res.*, 1989, **49**, 1682–1686.
10. Kelley, E. E., Buettner, G. R. & Burns, C. P. Production of lipid-derived free radicals in L1210 murine leukemia cells is an early oxidative event in the photodynamic action of photofrin. *Photochem. Photobiol.*, 1997, **65**, 576–580.
11. Miller, A. C. & Henderson, B. W. The influence of cellular glutathione content on cell survival following photodynamic treatment in vitro. *Radiat. Res.*, 1986, **107**, 83–94.
12. Jiang, F., Lilge, L., Belcuig, M., Singh, G., Greiner, J., Li, Y. & Chopp, M. Photodynamic therapy using Photofrin in combination with buthionine sulfoximine to treat 9L gliosarcoma in rat brain. *Lasers Surg. Med.*, 1998, **23**, 161–166.
13. Bailey, H. H. L-S,R-buthionine sulfoximine: Historical development and clinical issues. *Chem. Biol. Interact.*, 1998, **111–112**, 239–254.
14. Moan, J. Effect of bleaching of porphyrin sensitizers during photodynamic therapy. *Cancer Lett.*, 1986, **33**, 45–53.
15. Chekulayev, V., Shevchuk, I. & Chekulayeva, L. Photooxidation of hematoporphyrin derivative–bovine serum albumin complex. *Proc. Estonian Acad. Sci. Chem.*, 1997, **46**, 168–185.
16. Rotomskis, R., Bagdonas, S. & Streckyte, G. Spectroscopic studies of photobleaching and photoproduct formation of porphyrins used in tumour therapy. *J. Photochem. Photobiol. B: Biol.*, 1996, **33**, 61–67.
17. Chekulayev, V., Shevchuk, I. & Chekulayeva, L. Effects of light exposure on the uptake and destruction of hematoporphyrin derivative in Ehrlich carcinoma cell suspension. *Proc. Estonian Acad. Sci. Chem.*, 1998, **47**, 73–91.
18. Mang, T. S., Dougherty, T. J., Potter, W. R., Boyle, D. G., Somer, S. & Moan, J. Photobleaching of porphyrins used in photodynamic therapy and implications for therapy. *Photochem. Photobiol.*, 1987, **45**, 501–506.
19. Lipson, R., Baldes, E. & Olsen, A. The use of a derivative of hematoporphyrin in tumor detection. *JNCI*, 1961, **26**, 1–8.
20. Kessel, D., Thompson, P., Musselman, B. & Chang, C. K. Chemistry of hematoporphyrin-derived photosensitizers. *Photochem. Photobiol.*, 1987, **46**, 563–568.
21. Lötjönen, S. & Hynninen, P. H. A convenient method for the preparation of chlorin e_6 and rhodin g_7 trimethyl esters. *Synthesis*, 1980, 7, 541–543.
22. Chekulayev, V., Shevchuk, I., Mihkelsoo, V. & Kallikorm, A. A change in the sulfhydryl status of Ehrlich carcinoma cells by exposure to light in the presence of chlorin-e_6 trimethyl ester. The effect of L-buthionine-[S,R]-sulfoximine on the photosensitized damage of tumor cells. *Proc. Estonian Acad. Sci. Chem.*, 1992, **41**, 52–61.
23. Mossmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 1983, **65**, 55–63.
24. Chekulayev, V., Shevchuk, I. & Chekulayeva, L. The changes in the optical properties of Ehrlich carcinoma cells treated with hematoporphyrin derivative and light. In *23rd Estonian Chemistry Days, 1997, Tallinn, Book of Abstracts*. Tallinn, 1997, 24.
25. Samal, A. B., Cherenkevich, S. N. & Khmara, N. F. Identification of native actin in thrombocytes by fluorescence method. *Biofizika*, 1983, **28**, 298–301 (in Russian).
26. Chekulayev, V., Shevchuk, I., Chekulayeva, L. & Kahru, A. Increasing the efficiency of photosensitized damage of Ehrlich ascites carcinoma cells using haematoporphyrin derivative or chlorin-e_6 in combination with Lonidamine. *Proc. Estonian Acad. Sci. Biol.*, 1996, **45**, 102–127.
27. Chekulayev, V., Shevchuk, I., Mihkelsoo, V. & Kallikorm, A. Influence of metronidazole and some electron acceptors on the chlorin-e_6 photosensitized killing of Ehrlich carcinoma cells. In *Optical Methods for Tumor Treatment and Detection* (Dougherty, T. J., ed.). *Proc. SPIE*, 1992, **1645**, 131–140.

28. O'Dwyer, P. J., Hamilton, T. C., LaCreta, F. P., Gallo, J. M., Kilpatrick, D., Halbherr, T., Brennan, J., Bookman, M. A., Hoffman, J., Young, R. C., Comis, R. L. & Ozols, R. F. Phase I trial of buthionine sulfoximine in combination with melphalan in patients with cancer. *J. Clin. Oncol.*, 1992, **14**, 249–256.

L-BUTIONIIN-[S,R]-SULFOKSIMIINI MÕJU KASVAJARAKKUDE FOTOSENSIBILISEERITUD HÄVIMISELE HEMATOPORFÜRIINI DERIVAADI TOIMEL ILMA RAKUSISESE GLUTATIOONI LANGUSETA

Ljudmila TŠEKULAJEVA, Igor ŠEVTŠUK, Raissa JÄÄLAID ja Vladimir TŠEKULAJEV

Hematoporfüriini derivaati (HpD) kasutatakse laialdaselt fotosensibilisaatorina (FS) pahaloomuliste kasvajate fotodünaamilises teraapias (FDT). L-butioniin-[S,R]-sulfoksimiini (L-BSO) manustamine (koos HpD-ga) 5 minutit enne kiiritamist (kiirgusriba 590–830 nm, $\lambda_{max} = 630$ nm) Ehrlichi astsiitse kartsinoomi (EAK) rakkudele suurendas sünergistlikult HpD-ga sensibiliseeritud rakkude fotoinaktivatsiooni. 2,5 mM BSO kontsentratsiooni puhul suurenes oluliselt (ligi 20%) EAK rakkude HpD fototoksiline toime. Kuid seda BSO stimuleerivat efekti ei täheldatud, kui FS-ina kasutati kloriin-e_6-trimetüülestrit. BSO stimuleeriv toime HpD–FDT puhul ei olnud seotud redutseeritud glutatiooni (loomulik antioksüdant) sisalduse vähendamisega EAK rakkudes. Samas suurendas L-BSO kontsentratsioonil 0,5–2,5 mM HpD akumulatsiooni taset rakkudes nõrgalt, kuid statistiliselt tõeselt. Seda võiks seletada BSO stimuleeriva mõjuga HpD–FDT-le.