

Photosensitizers and antioxidants: a way to new drugs?†

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Photodynamic therapy (PDT) is a relatively new modality of treatment of diseases involving uncontrolled cell proliferation. It is based on the production of reactive species upon illumination of a photosensitizer in the presence of oxygen. Antioxidants are primarily reducing agents prone to scavenge reactive species in one way or another. Their presence in photodynamic reactions usually reduces the efficacy of PDT. Some antioxidants like ascorbic acid, α -tocopherol or butyl-4-hydroxyanisole, however, when added to cells at adequate concentrations may enhance the photodamaging activity of PDT. The presence of transition metals and precise timing of antioxidant administration may also be important factors in increasing the efficacy of PDT. Antioxidant carrier sensitizers have been designed, synthesised and tested for their antibacterial PDT activity. The promising results raise the question whether the introduction of antioxidant moieties into sensitizer molecules would lead to the synthesis of highly effective new drugs.

Introduction

Photodynamic therapy is a promising new treatment for diseases involving cell proliferation. It induces a highly complex series of transient changes in cells, involving reactive oxygen species (ROS). The basic mechanism involves the action of light in the

presence of a sensitizer and molecular oxygen, which generates the production of a series of ROS, mainly singlet oxygen and superoxide anion. The basic scheme of this reaction is represented in Fig. 1. Following the absorption of light, the sensitizer undergoes a transition from its ground state (S) into an electronically excited one (singlet $^1S^*$ to triplet $^3S^*$ state). Different reactions can take place from here on, but the triplet state mainly undergoes two kinds of reactions:¹ it can form

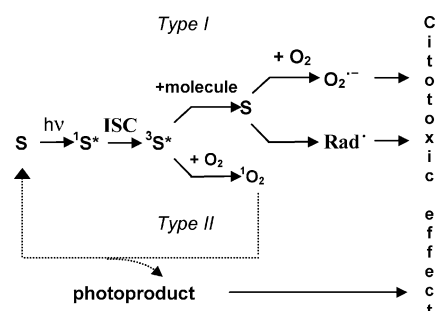


Fig. 1 Possible mechanisms of PDT effect. Light absorption ($h\nu$) causes the ground state sensitizer (S) to get into an electronically excited singlet state ($^1S^*$), which after an efficient intersystem crossing results in the triplet excited state ($^3S^*$) and subsequent electron or energy transfer to environmental oxygen, thereby producing free radicals (Type I reactions) or singlet oxygen (Type II reactions). ROS, mainly 1O_2 , could result in oxidative photoactivation of the sensitizer, and the resultant photoproducts may contribute to the observed cytotoxic effect.

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radicals ($O_2^{\cdot-}$, Rad *) after directly reacting with the substrate by hydrogen atom or electron transfer (Type I mechanism), or it can transfer its energy to molecular oxygen by directly reacting to form singlet oxygen (1O_2 , Type II mechanism). Type I and II reactions may occur simultaneously, and the ratio between the two processes highly depends on the sensitizer, substrate and oxygen concentration. Chemically reactive 1O_2 produced during conventional PDT could result in oxidative photoactivation of the sensitizer, and the resultant photoproducts may contribute to the observed cytotoxic effect.² In any case, photoactivation of the sensitizer renders the production of potentially lethal reactive species triggering photodynamic damage. Thus, the efficacy of PDT is mainly dependent on the *in situ* generation of ROS upon photo-exposure of the sensitizer.

Antioxidants, on the other hand, are powerful reducing agents and have the function to scavenge free radicals. Consequently, antioxidants could compete with oxygen for quenching of the triplet sensitizer or can neutralise the generated reactive species, thus counteracting the effect of PDT.

Effect of exogenous antioxidants on PDT

There are several reports on the protective effect of certain antioxidants against the lethal oxidative stress induced by photosensitization.³⁻⁸

Although known for their protective properties, antioxidants can exhibit pro-oxidant activity, especially in the presence of catalytic metals.⁹ Several research groups have reported results on the PDT enhancing activities of certain antioxidant molecules, such as α -tocopherol,¹⁰ Trolox ((\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a water soluble vitamin E analogue),¹¹ ascorbate¹²⁻¹⁷ and 3(2)-*tert*-butyl-4-hydroxyanisole (BHA).¹⁸⁻¹⁹ Different sensitizers and different tumour models were used in these studies suggesting that the observed phenomenon is not limited to a particular system.

First, Girotti and coworkers^{12,13} have shown that photodamage to lipids and erythrocyte membranes can be enhanced by the addition of ascorbate to non-bound uroporphyrin- and membrane-bound protoporphyrin-mediated PDT. Rosenthal and Benhur¹⁴ investigated the rate of photohaemolysis of human red blood cells sensitised by chloroaluminium phthalocyanine sulfonate and found that it was increased by ascorbate, with or without added iron salt. Lysis rate and ascorbate concentration were directly related, suggesting that ascorbate acted as a reactant and not as a catalyst. Measurements indicated some formation of singlet oxygen, although to a lesser extent than during the photosensitised haemolysis in the absence of ascorbate. They concluded that parallel to the singlet oxygen-mediated process, a fraction of the photodynamic damage proceeded *via* Type I, ascorbate-assisted, mechanism.

Buettner and his coworkers¹⁵ investigated the photodynamic effect of Photofrin[®] as sensitizer on the growth of L1210 and SCC-25 tumour cells in the presence of Fe(II) and 100 μ M ascorbic acid. They found that the combination of the metal ion with ascorbic acid increased the cytotoxic effect of *in vitro* PDT through elevated lipid hydroperoxide formation. Later, Kelley *et al.*¹⁶ described that the increase in lipid peroxidation was two-fold when the sensitizer was administered to cells before PDT and five-fold when administered after 5 min of illumination of SCC-25 cells. The authors explained their findings by the formation of lipid peroxides during Photofrin-mediated PDT. The presence of ascorbate served to reduce Fe(III) to Fe(II), which donates an electron to the formed peroxides, thereby initiating free radical chain reactions enhancing the cytotoxicity of PDT. Somewhat similar results were described by Kaliya *et al.*¹⁷ They found that Photosens (the Russian version of tetra-4-*tert*-butyl-phthalocyanine derivative) was less phototoxic in several murine tumour models in the presence of low doses of ascorbate and more phototoxic at high concentrations (100 mg kg⁻¹ of

ascorbate). These data support the concentration-dependent activity of ascorbic acid during *in vivo* PDT.

In 1997, it was found that another antioxidant, BHA enhanced the photodynamic formation of the oxidised product of DNA damage (8-oxo-7,8-dihydro-2'-deoxyguanosine) in cultured liver cells after exposure to lomefloxacin and UVA irradiation.¹⁸ Shevchuk *et al.*¹⁹ found using hematoporphyrin derivative (HpD) as sensitizer that high concentrations of BHA (0.56 mM) in EAC cells had a PDT enhancing effect, while at lower concentrations (0.14–0.28 mM) the same antioxidant was ineffective. It had a three-fold increasing effect during *in vitro* PDT and a ten-fold increasing effect when administered to cells right after PDT. The PDT-enhancing activity of 0.5 mM BHA on death of EAC cells was similar when measured in the ascitic fluid *in vivo*. When BHA was given to tumour-bearing mice immediately after HpD-PDT, the combination was found to be three to four times more effective than when BHA was added to EAC cells prior to light exposure. It has been previously shown that BHA may inhibit the oxygen consumption in tumour cells by blocking the electron flow in the mitochondria, thus inhibiting oxidative phosphorylation and ATP synthesis,²⁰ which leads to diminished cellular activities. In mice bearing Ehrlich ascites tumour, the authors found a good correlation between the concentration of BHA that increased the efficiency of HpD-PDT and the concentration that inhibited the oxygen consumption and dehydrogenase activity of tumour cells. Therefore, it has been suggested that the potentiating effect of BHA on PDT could be caused by the impairment of mitochondrial respiration, and the difference in its action on the efficiency might be explained by the ability of the antioxidant to inhibit the photosensitised destruction of some biomolecules.

Melnikova *et al.*¹⁰ have shown in the colon carcinoma HT29 cell line that 0.33–1 mM of α -tocopherol can enhance PDT activity of *meta*-tetra(hydroxyphenyl)chlorin (*m*THPC) in cell culture, while lower concentrations of the antioxidant (0.001–0.1 mM) had no significant effect in the same system. Under the same conditions, α -tocopherol was not incorporated and did not affect *m*THPC-sensitised photokilling of normal fibroblasts. A similar effect was observed *in vivo*, when Trolox, an α -tocopherol analogue, was injected into nude mice bearing HT29 human adenocarcinoma xenografts before *m*THPC-PDT.¹¹ Trolox must have been present in the photochemical stage to improve tumour response to PDT since its injection after irradiation was ineffective. Laserflash photolysis measurements demonstrated that free radicals were formed in deoxygenated methanolic solution of *m*THPC in the presence of Trolox suggesting that a shift from Type II reaction toward radical producing (Type I) processes was occurring, probably due to oxygen depletion in tumours. Then, the phototoxicity of *m*THPC may be derived from the reaction of the sensitizer radical (*m*THPC $^{\cdot-}$) with oxygen, leading to generation of $O_2^{\cdot-}$, which could produce other ROS, and from the simultaneously formed Trolox radical (Trol $^{\cdot}$ = Rad *).

We have tested in our laboratory the effect of a series of unrelated antioxidant molecules on pheophorbide *a* (Phe *a*)-PDT in S180 murine sarcoma cell line. Fig. 2 shows that in our *in vitro* system the effect of the antioxidant strongly depended on the nature of the added molecule²¹. Resveratrol (a polyphenol) and quercetin (a flavonoid) protected cells from the phototoxic effect of Phe *a*-PDT, ascorbic acid at 1 and 10 μ M concentrations had no effect, while *N* acetyl-L-cysteine (*N*-Ac cysteine) and Trolox enhanced the efficacy of cell photoinactivation at concentrations as low as 1–100 μ M. Quercetin, which had no PDT enhancing effect, had no influence on the production of H_2O_2 and steady state free radicals in S180 cells measured right after PDT, while the PDT enhancing *N*-Ac cysteine significantly increased the levels of both measured parameters (Fig. 3). These data are in accordance with the theory that some, but not all antioxidants act as pro-oxidants under certain circumstances.

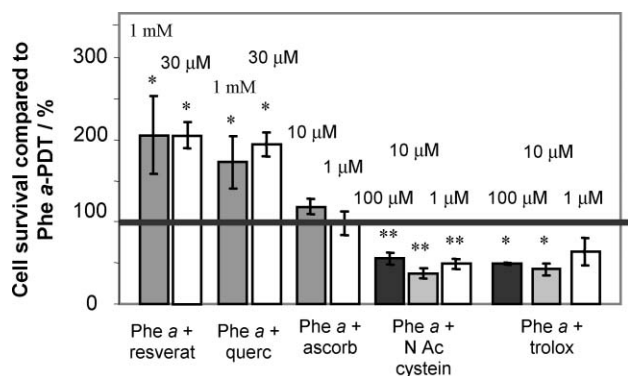


Fig. 2 PDT activity of pheophorbide *a* (Phe *a*, 300 nM) on S180 sarcoma cells in the presence of different antioxidants, expressed as percentage of cell survival compared to Phe *a*-PDT (100%). Results are expressed as means \pm SD. (*) $p < 0.05$ and (**) $p < 0.07$ compared to Phe *a*-PDT, based on Student's *t* test ($n = 4-6$).

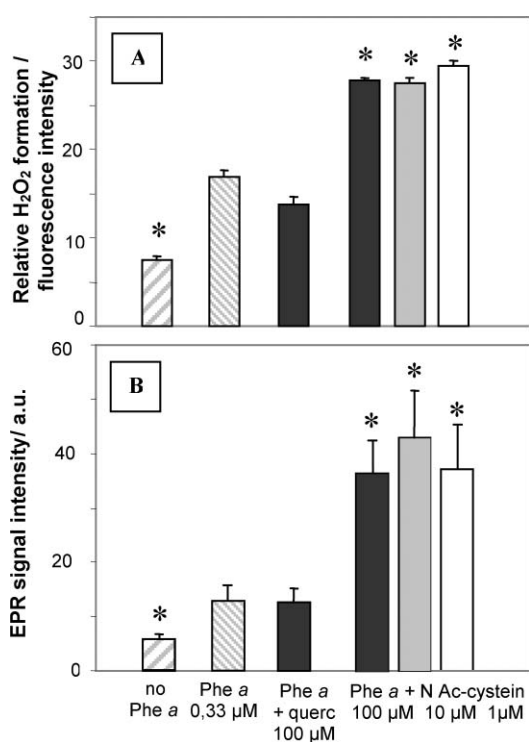


Fig. 3 Steady state concentration of free radicals measured by electron spin resonance spectroscopy, ESR (A) and hydrogen peroxide formation measured by dichlorofluorescein-fluorescence (B) in S180 cells after 300 nM of Phe *a*-PDT in the presence of quercetin or different concentrations of *N* acetyl cysteine. Results are expressed as means \pm SD. (*) $p < 0.05$ compared to Phe *a*-PDT, based on Student's *t* test ($n = 4-6$).

It is interesting to note that nitric oxide ($\cdot\text{NO}$) can also act as antioxidant in biological systems protecting COH-BR1 breast tumour cells from 5-aminolevulinic acid-induced photodynamic damage.²² Nevertheless, at high concentrations $\cdot\text{NO}$ can sensitise cells to anticancer agents that possess an oxidative mechanism of action.²³ The effect could be due to affecting the cellular antioxidant enzyme profile or other unknown mechanism of action.

In conclusion, antioxidant molecules under certain circumstances can protect or sensitise cells to PDT. The effect does not seem to depend on the nature of sensitizer, but rather on the structure of the antioxidant and mainly on the circumstances of its action. The concentration of the antioxidant seems to be a major factor as well. In general, compounds such as carotenoids, tocopherols or ascorbate derivatives will demon-

strate an antioxidant or pro-oxidant characteristic depending on the redox potential of the individual molecule, the inorganic chemistry of the cell and the cellular oxygen environment.²⁴ Antioxidant molecules can serve as substrates for $\text{S}^{\cdot-}$ -mediated reactions, producing antioxidant radicals in the process of either radical scavenging reaction²⁵ or spontaneous auto-oxidation.²⁶ Antioxidants can also act as pro-oxidants during PDT, especially under hypoxic conditions. The main effector of PDT under usual conditions favours Type II mechanism. PDT treatment leads to oxygen depletion in tumour tissue shifting the contribution of singlet oxygen-mediated process (Type II reaction) toward free radical production (Type I reaction).¹¹ The possible mechanism of antioxidant-mediated free radical generation during PDT is depicted in Fig. 4. A pro-oxidant pathway can be due to the photochemical conversion of the sensitizer to its triplet excited state ($^3\text{S}^*$), which yields the formation of sensitizer radical anion ($\text{S}^{\cdot-}$) and antioxidant radical (Antiox \cdot^*). These free radicals may react with residual oxygen leading to the formation of superoxide radical anion or other ROS. Therefore, the Antiox \cdot^* -mediated pathway can work in relay with $^1\text{O}_2$ while oxygen concentration is decreased in the course of PDT, shifting Type II mechanism toward Type I.

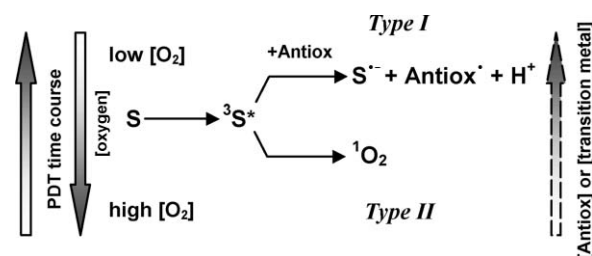


Fig. 4 Possible mechanism of PDT effect in the presence of pro-oxidants. Singlet oxygen reaction (Type II) is dominant at the onset of illumination in the presence of a sensitizer (S). Under hypoxic conditions production of singlet oxygen decreases with the concomitant increase of the radical pathway (Type I) mediated by the antioxidant, producing sensitizer anion ($\text{S}^{\cdot-}$) and antioxidant (Antiox \cdot^*) radicals. Increasing antioxidant or transitional metal concentrations in cells shifts the reaction toward Type I mechanism.

Effect of transition metals

Trace levels of transition metals can participate in the production of free radicals, thereby increasing the pro-oxidant chemistry of these metals. Buettner and coworkers^{9,16,27} and others^{12,26,28} have discussed this issue in PDT. Singlet oxygen generated in the course of a Type II reaction may react with membrane lipids to form lipid hydroperoxides, which lead to the formation of oxidising free radicals. The latter reaction can be enhanced by the presence of catalytic transition metals (mainly iron and copper in cells) forming highly oxidising lipid alkoxy radicals. Kelley *et al.*¹⁶ hypothesised that transition metal ions in the presence of ascorbate could serve as pro-oxidants in a photosensitizer-mediated reaction. It is well known that free transition metal ions can be found at extremely limited concentrations in cells, if at all, under normal conditions, as they are bound to proteins. Nevertheless, it has been reported for example that UV light exposure²⁹ of skin results in an increase of non-protein bound iron, suggesting that irradiation may result in free transition metal ion release, leading to additional photodamage during PDT. We are currently investigating this possibility using iron- and copper-chelating agents during PDT treatment of cells.

Effect of concentration and timing of antioxidant addition

Concentration and timing of antioxidant addition to cells seems to be a major factor regarding the outcome of the treatment as well. Some antioxidants have a concentration-dependent

influence on PDT efficacy when added to photosensitized cells. The aforementioned studies of Melnikova *et al.*¹⁰ and Shevchuk *et al.*¹⁹ showed that antioxidants at low concentrations had no effect in cell culture, while higher concentrations of the same antioxidants significantly enhanced the efficacy of PDT. The PDT-enhancing effect of ascorbic acid was also concentration-dependent in several murine tumours.¹⁷ Apparently, the presence of higher antioxidant concentrations in cells shifts the mechanism of PDT action from ¹O₂-mediated process toward a predominantly free-radical-mediated one. This is supported by the findings of Bachowski *et al.*²⁸ showing that irradiation of the photosensitizer MC540 produced significantly less primary ¹O₂-adducts and induced a large scale formation of free radical-mediated products in human erythrocyte ghosts in the presence of iron and 1 mM ascorbate.

Several antioxidants had no or little PDT effect when administered before illumination and had a much greater PDT-enhancing effect when administered right after the treatment. This was observed in *in vitro* reactions and under cell culture conditions,^{15,16,19} but could not be confirmed *in vivo*.¹¹ Probably, because the timing of antioxidant uptake into tumours is much more difficult to control in live animals than in cultured cells. Kinetic studies of antioxidant uptake into each particular tumour tissue should be performed before testing the effect of timing of antioxidant addition on PDT efficacy in living species.

A possible explanation for the importance of timing of antioxidant administration in achieving higher PDT efficacy is the pro- or antioxidant role that the additive plays during the reaction. Singlet oxygen reaction is dominant at the onset of irradiation when antioxidants, especially at low concentrations, may be used up as true reducing agents. With time, hypoxic conditions prevail and the contribution of free radical pathways increases. Then, leftover antioxidants would serve as pro-oxidants leading to the concomitant formation of ROS and antioxidant radicals. The later the antioxidant is added to the system, the more it is prone to act as pro-oxidant.

Design of antioxidant carrier sensitizers (ACS)

Based on these intriguing results, we have designed some ACSs, which are 4-oxy-3,5-di-*tert*-butylphenyl-,³⁰ as well as butyl hydroxy toluene- and propyl gallate-substituted hematoporphyrins. All these compounds had an enhanced antioxidant capacity when compared to Photofrin®.²¹ They also turned out to be effective photosensitizers against multidrug-resistant bacteria, especially against gram-positive species.³¹ The positive bacteria-killing effect was attributed in this case to the specific damage to different bacterial cell membrane ion pumps. A more detailed mechanism of action of ACS molecules remains to be elucidated.

To summarize, the absence of toxicity and mutagenic properties of most antioxidants applied at reasonable concentrations makes them ideal additives for the potentiation of PDT efficacy. The potential therapeutic gain of the application of certain antioxidants during or after PDT lies for example, in preventing side effects of PDT, like skin erythematous reactions.¹¹ A very important implication is that sensitizers can be applied at lower concentrations in conjunction with antioxidants to achieve the same or higher PDT efficacy, which is also expected to reduce treatment toxicity. The selection of antioxidant concentrations and the sequence of their addition are of extreme importance in taking advantage of the described results. Antioxidants are clinically authorized drugs, thus, their usage (perhaps in a topical combination with transition metals) may result in a simple and cheap way to improve several PDT protocols.

Introduction of antioxidant carrier sensitizers is a new idea showing promising results in fighting multiple drug-resistant bacteria. Testing of ACS compounds is under way in eukaryotic cell cultures and animal tumour models. Our preliminary data

lead us to speculate that the combination of photosensitizer molecules with antioxidant moieties could be a new way to improve the efficacy of certain sensitizers.

Precautions, however, should be taken when choosing the appropriate antioxidant. These molecules have been usually known to reduce the efficacy of PDT and there seems to be a fragile equilibrium between their effects in reducing or enhancing PDT-efficacy. Whether certain antioxidants demonstrate an antioxidant or pro-oxidant characteristic seems to depend on the structure of the antioxidant itself, the concentration and timing of antioxidant addition, *etc.* Since their addition can have different effects depending on several circumstances not fully understood yet, many preliminary experiments should be performed before deciding whether the addition of a given antioxidant or an ACS compound will result in "harmful" antioxidant or "helpful" pro-oxidant during PDT.

References

- 1 B. W. Henderson and T. J. Dougherty, How Does Photodynamic Therapy Work?, *Photochem. Photobiol.*, 1992, **55**(1), 145–157.
- 2 S. Pervaiz, Reactive oxygen-dependent production of novel photochemotherapeutic agents, *FASEB J.*, 2001, **15**, 612–617.
- 3 G. De Guidi, R. Chillemi, S. Giuffrida, G. Condorelli and M. Cambria, Fama, Molecular mechanism of drug photosensitization. Part 3. Photohemolysis sensitized by diflunisal, *J. Photochem. Photobiol., B*, 1991, **10**, 221–237.
- 4 J. P. Kamat and T. P. Devasagayam, Oxidative damage to mitochondria in normal and cancer tissues, and its modulation, *Toxicology*, 2000, **155**, 73–82.
- 5 A. R. Kamuhabwa, P. M. Agostinis, M. A. D'Hallewin, L. Baert and P. A. M. de Witte, Cellular photodestruction induced by hypericin in AY-27 rat bladder carcinoma cells, *Photochem. Photobiol.*, 2001, **74**, 126–132.
- 6 M. J. Jou, S. B. Jou, H. M. Chen, C. H. Lin and T. I. Peng, Critical role of mitochondrial reactive oxygen species formation in visible laser irradiation-induced apoptosis in rat brain astrocytes (RBA-1), *J. Biomed. Sci.*, 2002, **9**, 507–516.
- 7 C. Perotti, A. Casas and A. M. Del C Battle, Scavengers protection of cells against ALA-based photodynamic therapy-induced damage, *Lasers Med. Sci.*, 2002, **17**, 222–229.
- 8 J. C. Stockert and J. Herkovits, Photodynamic toxicity and its prevention by antioxidative agents in *Bufo arenarum* embryos, *Toxicology*, 2003, **192**, 211–218.
- 9 G. R. Buettner and B. A. Jurkiewicz, Catalytic metals, ascorbate and free radicals: combinations to avoid, *Radiat. Res.*, 1996, **145**, 532–541.
- 10 V. Melnikova, L. Bezdetsnaya, I. Belitchenko, A. Potapenko, J. L. Merlin and F. Guillemin, *Meta*-tetra(hydroxyphenyl)chlorin-sensitized photodynamic damage of cultured tumor and normal cells in the presence of high concentrations of α -tocopherol, *Cancer Lett.*, 1999, **139**, 89–95.
- 11 V. O. Melnikova, L. N. Bezdetsnaya, D. Brault, A. Y. Potapenko and F. Guillemin, Enhancement of *meta*-tetrahydroxyphenylchlorin-sensitized photodynamic treatment on human tumor xenografts using a water-soluble vitamin E analogue, trolox, *Int. J. Cancer*, 2000, **88**, 798–803.
- 12 A. W. Girotti, J. P. Thomas and J. E. Jordan, Prooxidant and antioxidant effects of ascorbate on photosensitized peroxidation of lipids in erythrocyte membranes, *Photochem. Photobiol.*, 1985, **41**, 267–276.
- 13 G. J. Bachowski, K. M. Morehouse and A. W. Girotti, Porphyrin-sensitized photoreactions in the presence of ascorbate: oxidation of cell membrane lipids and hydroxyl radical traps, *Photochem. Photobiol.*, 1988, **47**, 635–645.
- 14 I. Rosenthal and E. Benhur, Ascorbate-assisted, phthalocyanine-sensitized photohemolysis of human erythrocytes, *Int. J. Radiat. Biol.*, 1992, **62**, 481–486.
- 15 G. R. Buettner, E. E. Kelley and C. P. Burns, Membrane lipid free radicals produced from L1210 murine leukemia cells by Photofrin photosensitization: An EPR spin trapping study, *Cancer Res.*, 1993, **53**, 3670–3673.
- 16 E. E. Kelley, F. E. Domann, G. R. Buettner, L. W. Oberley and C. P. Burns, Increased efficacy of *in vitro* Photofrin® photosensitization of human oral squamous cell carcinoma by iron and ascorbate, *J. Photochem. Photobiol., B*, 1997, **40**, 273–277.

- 17 O. L. Kaliya, L. A. Lukyanets and G. N. Vorozhtsov, Catalysis and photocatalysis by phthalocyanines for technology, ecology and medicine, *J. Porphyrins Phthalocyanines*, 1999, **3**, 592–610.
- 18 J. E. Rosen, Proposed mechanism for the photodynamic generation of 8-oxo-7,8-dihydro-2'-deoxyguanosine produced in cultured cells by exposure to lomefloxacin, *Mutat. Res.*, 1997, **381**, 117–129.
- 19 I. Shevchuk, V. Chekulayev and L. Chekulayeva, Enhancement of the efficiency of photodynamic therapy of tumours by t-butyl-4-hydroxyanisole, *J. Photochem. Photobiol., B*, 1998, **45**, 136–143.
- 20 E. Fones, H. Amigo, K. Gallegos, K. Guerrero and J. Ferreira, T-Butyl-4-hydroxyanisole as an inhibitor of tumor cell respiration, *Biochem. Pharmacol.*, 1989, **38**, 3443–3451.
- 21 O. Farkas and J. Jakus, Effect of different antioxidants on the photodynamic effect of pheophorbide *a* in cultured tumor cells submitted.
- 22 M. Niziolek, W. Korytowski and A. W. Girotti, Chain-breaking antioxidant and cytoprotective action of nitric oxide on photodynamically stressed tumor cells, *Photochem. Photobiol.*, 2003, **78**, 262–270.
- 23 E. E. Kelley, C. J. Weydert, S. M. Martin, A. J. Fischer, L. W. Oberley and G. R. Buettner, Endogenously produced nitric oxide sensitizes cancer cells to photodynamic treatment, *Free Radical Biol. Med.*, 2001, **31**, S80, 238, Suppl. 1.
- 24 J. L. Schwartz, The dual roles of nutrients as antioxidants and prooxidants: Their effects on tumor cell growth, *J. Nutr.*, 1996, **126**, S1221–S1227.
- 25 V. W. Bowry and R. Stocker, Tocopherol-mediated peroxidation. The prooxidation effect of vitamin E on the radical-initiated oxidation of human low-density lipoprotein, *J. Am. Chem. Soc.*, 1993, **115**, 6029–6044.
- 26 E. T. Denisov, Realization of different mechanisms of inhibition of hydrocarbon oxidation by phenols, *Khim. Fiz.*, 1983, **2**, 229–238 (in Russian).
- 27 G. R. Buettner, Ascorbate autoxidation in the presence of iron and copper chelates, *Free Radical Res. Commun.*, 1986, **1**, 349–353.
- 28 G. J. Bachowski, T. J. Pintar and A. W. Girotti, Photosensitized lipid peroxidation and enzyme inactivation by membrane-bound merocyanine 540: reaction mechanisms in the absence and presence of ascorbate, *Photochem. Photobiol.*, 1991, **53**, 481–491.
- 29 D. L. Bissett, R. Chatterjee and D. P. Hannon, Chronic ultraviolet radiation-induced increase in skin iron and the photoprotective effect of topically applied iron chelators, *Photochem. Photobiol.*, 1991, **54**, 215–223.
- 30 A. V. Melezhik and V. D. Pochodenko, Tetra(4-oxi-3,5-di-*tert*-butylphenyl)porphyrin and the paramagnetic characteristics of its derivatives, *Zh. Org. Khim.*, 1982, **XVIII**, 1054–1059 (in Russian).
- 31 H. Ashkenazi, Y. Nitzan and D. Gal, Photodynamic effects of antioxidant substituted porphyrin photosensitizers on gram-positive and negative bacteria, *Photochem. Photobiol.*, 2003, **77**, 186–191.