Oxidative Photodamage Induced by Photodynamic Therapy with Methoxyphenyl Porphyrin Derivatives in Tumour-Bearing Rats

Abstract. The oxidative effects of photodynamic therapy with 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (TMP) and Zn-5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (ZnTMP) were evaluated in Wistar rats subcutaneously inoculated with Walker 256 carcinoma. The animals were irradiated with red light (λ = 685 nm; D = 50 J/cm²; 15 min) 3 h after intra-peritoneal administration of 10 mg/kg body weight of porphyrins. The presence of free radicals in tumours after photodynamic therapy with TMP and ZnTMP revealed by chemiluminescence of luminol attained the highest level at 18 h after irradiation. Lipid peroxides measured as thiobarbituric-reactive substances and protein carbonyls, which are indices of oxidative effects produced on susceptible biomolecules, were significantly increased in tumour tissues of animals 24 h after photodynamic therapy. The levels of thiol groups and total antioxidant capacity in the tumours were decreased. The activities of antioxidant enzymes superoxide dismutase and glutathione peroxidase were also increased in tumour tissues after photodynamic therapy. Increased levels of plasma lipid peroxides as well as changes in the levels of erythrocyte antioxidant enzyme activities suggest possible systemic effects of photodynamic therapy with TMP and ZnTMP.

Introduction

Photodynamic therapy (PDT) is an alternative non-invasive treatment, which has been applied in several solid malignancies as well as non-oncological diseases. This type of treatment involves systemic or topical application of a photosensitive substance (PS) or a metabolic precursor for such a substance, followed by local irradiation with light in visible domain. As a consequence, a photochemical reaction leading to singlet oxygen (³O₂) and other reactive oxygen species (ROS) generation in targeted tissues takes place. These species initiate oxidation of susceptible biomolecules, leading to cell destruction via necrosis and/or apoptosis (Dougherty et al., 1998).

A large number of substances have been tested in vitro and in vivo in PDT experiments. Biological effects of PS largely depend on their physicochemical properties: physical and chemical stability, short-time interval between administration and maximal accumulation in tumour tissue, rapid clearance from the body, low toxicity, optimal tissue penetration of the light at the wavelength producing activation, minimal pharmacological interactions with other drugs used in oncological treatments (Jori, 1996).

The most extensively used photosensitizers used in PDT are porphyrin derivatives. They fulfil many of the requirements mentioned above. The development of new compounds with higher affinity for cancer cells and the need for shorter times for sensitization are subjects of research at present (Jori, 1996; Nowis et al., 2005).

Physicochemical properties of a series of 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrins have shown that these synthetic compounds could be used as efficient PS. In vitro these agents photoinduced a cytotoxic effect on the Hep-2 human larynx carcinoma cell line (Milanesio et al., 2001).
In the present work, the dynamics of reactive oxygen species (ROS) production after PDT with 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (TMP) and Zn-5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (ZnTMP) together with the oxidative effects on biomolecules and antioxidant systems in tumour tissues and blood from Wistar rats bearing Walker 256 carcinosarcoma were investigated.

Material and Methods

Chemicals

TMP (5,10,15,20-tetrakis(4-methoxyphenyl)-21H,23H porphyrin) and ZnTMP (Zn-5,10,15,20-tetrakis(4-methoxyphenyl)-21H,23H porphyrin) were prepared in IICECHIM (Bucharest, Romania) laboratories by professor R. M. Ion and have been characterized (Ion et al., 1993, 1996-1997, 1997, 2007). Luminol, cytochrome c, xanthine, xanthine oxidase, β-nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPH), glutathione reductase, glutathione reduced, 2,4-dinitro-phenylhydrazine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate (ABTS), 1,1,3,3-tetraethoxypropane were from Sigma-Aldrich (Sigma–Aldrich Chemicals GmbH Inc., Seelze, Germany); 2-thiobarbituric acid, 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), guanidine hydrochloride, dimethyl sulphoxide (DMSO) were purchased from Merck KgA (Darmstadt, Germany) and KH₂PO₄, K₂HPO₄, Na₂SO₄, ethanol and KH₂PO₄, K₂HPO₄. 3H₂O, Na₂SO₄ were from Chimopar (Bucharest, Romania). All the reagents were of analytical grade.

Animals and tumours

The experiments were performed in 46 male Wistar rats (180 ± 20 g) supplied by the Animal Department of “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca. They were maintained in the animal house facility of the Department of Physiology in order to accommodate for seven days. Rats were fed standard laboratory pellets and received water ad libitum. All the experiments were performed in accordance with protocols approved by the experimental animal welfare committee of the “Iuliu Hatieganu” University.

For in vivo tumour propagation, small fragments of Walker 256 tumours were subcutaneously (s.c.) transplanted to recipient experimental rats in the right thigh under anaesthesia. Tumour-bearing rats were used for PDT studies when the mean tumour volume reached 1 cm³.

PDT studies

Tumour-bearing animals were intra-peritoneally (i.p.) injected with 10 mg/kg b.w. TMP or ZnTMP dissolved in DMSO. Twenty-four hours after drug administration the animals were anaesthetized (90 mg/kg ketamine, 10 mg/kg xylasine cocktail i.p.), shaved, and the skin above the tumour in the thigh was laser-irradiated for 15 min.

The Laser Therapeutic model D-68 was used as light source (λ = 685 nm) (dose: 50 J/cm², mean power: 25 W, frequency: 10 Hz). Untreated (control) and irradiated without porphyrin treatment rats served as comparison groups.

Chemiluminescence measurements

At 15 min, 30 min, 2 h, 6 h, 18 h and 24 h intervals after irradiation, three animals from each TMP- and ZnTMP-treated groups were sacrificed. Tumours were removed and washed with cold phosphate-buffered saline. Small fragments were transferred into phosphate buffer 50 mM, pH 7 containing 0.2 mM luminol at 37 °C. Chemiluminescence (CL) was recorded for 5 min with an LS45 Perkin Elmer spectrophotometer. Area under the curve (AUC) was calculated and expressed as arbitrary light units/min/mg wet tissue (Uneri et al., 2006).

Biochemical assays

Biochemical assays were performed in tumours and blood samples taken from TMP- and ZnTMP-treated groups as well as untreated or irradiated controls. Each group consisted of seven rats. Twenty-four hours after irradiation venous blood samples were collected on heparin. Thereafter, animals were sacrificed and tumours were removed. Blood was centrifuged at 1000 g for 5 min to separate the plasma. Erythrocytes were washed three times in cold saline and haemolysed by adding ice-cold ultrapure water in order to obtain lysates. Homogenates from tumour tissue fragments were prepared in 50 mM TRIS – 10 mM EDTA buffer (pH 7.4) with a Polytron homogenizer. Supernatants were obtained after homogenate centrifugation at 1500 g. Plasma, erythrocyte lysates, as well as homogenate supernatants from each group of animals were stored in aliquots at -80 °C until assayed.

TBARS determination

For the determination of thiobarbituric-reactive substances (TBARS), proteins from homogenates or plasma were precipitated with 20% trichloroacetic acid and then separated by centrifugation. The supernatant was treated with a solution of 2-thiobarbituric acid (0.67%) in 2 M HCl for 1 h at room temperature in the dark. After cooling, the absorbance of the total TBARS was measured at 530 nm. Tetra-ethoxypropane was used as a standard (1–10 μM). Concentration of TBARS was expressed in terms of malondialdehyde (MDA): nmol/mg protein for homogenates or nmol/ml for plasma (Esterbauer and Chjeeseman, 1994).

Protein carbonyls

For the assessment of protein carbonyls (PC), the reaction with 2,4-dinitrophenyl-hydrazine was used (Reznick and Packer, 1994). The samples were treated with a solution of 10 mM 2,4-dinitrophenyl hydrazine in 2.5 N HCl for 1 h at room temperature in the dark. After precipitation of protein with 20% trichloroacetic acid...
and centrifugation, the washed protein pellet was dissolved in 6 M guanidine hydrochloride. The absorbance was read at 355 nm and the carbonyl content was determined using the absorption coefficient for aliphatic hydrazones (22,000 M\(^{-1}\)cm\(^{-1}\)). The final concentration of proteins in each sample was estimated by readings at 280 nm using a standard curve obtained with bovine serum albumin standards. PC values were expressed as nmol carbonyl/mg protein.

*Protein thiol* group measurement was performed using the 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB or Ellman reagent) method (Hu, 1994). Samples were diluted with 0.25 M TRIS – 20 mM EDTA buffer, pH 8.2. The absorbance at 412 nm was determined before and 15 min after the treatment with 10 mM DTNB in methanol solution. The total concentration of thiol groups was calculated using an absorption coefficient of 13,600 M\(^{-1}\)cm\(^{-1}\). Concentrations of -SH groups were expressed as μmol/mg protein or μmol/ml.

Antioxidant capacity was determined using the ABTS test. In order to form the reagent containing ABTS ′, 2.45 mM potassium persulphate (final concentration) was added to a solution of 7 mM ABTS and left in the dark for 12 h. This reagent was diluted with phosphate-buffered saline, pH 7.4 to obtain a working solution presenting absorption of 0.70 ± 0.02. The decolourization of the working solution was recorded 6 min after the addition of the unknown sample. A concentration-response curve for percent inhibition of absorbance at 734 nm of ABTS ′ solution as a function of standard Trolox solutions was used to evaluate the antioxidant capacity. Results were expressed as eq. μmol Trolox/mg protein for homogenate or eq. μM Trolox for plasma (Re et al, 1999).

Superoxide dismutase activity was determined using the cytochrome c reduction test (Beauchamp and Fridovich, 1971). Briefly, homogenate or erythrocyte lysates were introduced into a cytochrome c solution (2 μM in 50 mM phosphate buffer, pH 7.8) containing xanthine (5 μM). The reaction was started by adding xanthine oxidase (0.2 U/ml in 0.1 mM EDTA). The increasing absorbance at 550 nm, indicating cytochrome c reduction, was recorded for 5 min. One unit of SOD inhibits the rate of increase in absorbance at 550 nm by 50 % of that produced for a control sample without SOD under the conditions of the assay. Results were expressed in U/mg protein.

Catalase activity in homogenates and lysates was measured in a reaction mixture containing 10 mM hydrogen peroxide in 50 mM kalium phosphate buffer, pH 7.4. The enzyme quantity which produced an absorbance reduction of 0.43 at 25° per minute at 240 nm in this system was defined as one unit of catalase activity. The activity was expressed as unit/mg protein (Pippenger et al., 1998).

Glutathione peroxidase activity was determined using an indirect method measuring the rate of disappearance of NADPH adapted in the laboratory. The reaction mixture consisted of 1 mM GSH, 0.24 U/ml glutathione reductase and 0.15 mM NADPH (final concentrations) in 50 mM phosphate buffer, pH 7.0. Appropriate volumes of samples were added and the reaction mixture was incubated at 37° for 5 min. The assay was initiated with t-butyl hydroperoxide solution (12 mM). The decrease in absorbance at 340 nm was recorded for 3 min. GPx activity was expressed as nmols NADPH consumed/ min/mg protein and were calculated using 6.2 × 10\(^{-6}\) molal absorptivity for NADPH (Flohe and Gunzler, 1994).

Protein levels in tumour tissues were evaluated with Bradford method (Noble and Bailey, 2009).

**Results**

**Evaluation of luminescence**

In order to detect the presence of free radicals after PDT with TMP and ZnTMP, the luminal-enhanced luminescence levels were measured at different time intervals in animal tumours. The chemiluminescence increased steadily reaching a 3-fold higher level than those of controls at 18 h interval. Afterwards, a decline of luminescence was observed at 24 h in both treated groups (Fig.1).

**Evaluation of lipid peroxides**

After 24 h from PDT the level of lipid peroxides evaluated as TBARS in tumours increased in both TMP and ZnTMP PDT-treated animals. The level of TBARS was significantly higher in TMP group (P < 0.02) and ZnTMP group (P < 0.042) as compared with the control group (Fig. 2A). The TBARS level was also significantly increased in the plasma of PDT-treated rats (Table 1). Kruskall-Wallis test, which evaluated the overall difference between the groups, showed a significant difference (P < 0.015) both in tumours and in the plasma.

**Evaluation of protein carbonyls**

Protein oxidative damage as reflected by carbonyl groups in the proteins after PDT was present in tumours. The protein carbonyls were significantly increased in tumour tissues after PDT with TMP and ZnTMP, the level of TBARS was significantly increased in both TMP and ZnTMP PDT-treated animals. The level of TBARS was significantly higher in TMP group (P < 0.02) and ZnTMP group (P < 0.042) as compared with the control group (Fig. 2A). The TBARS level was also significantly increased in the plasma of PDT-treated rats (Table 1). Kruskall-Wallis test, which evaluated the overall difference between the groups, showed a significant difference (P < 0.015) both in tumours and in the plasma.

**Evaluation of thiol groups**

Thiol groups act as antioxidants. Their levels are indicative of oxidative damage produced in proteins by ROS. The concentration of thiol groups was strongly reduced in tumour tissues of porphyrin-treated groups vs. control 24 h after PDT (P < 0.01) (Fig. 3A). Thiol pas-
ma levels were slightly increased, although not significantly as compared with the controls (Table 1).

**Evaluation of antioxidant capacity**

The antioxidant capacity of tumour tissues evaluated with the ABTS test was significantly impaired in animals after PDT (P = 0.01) (Fig. 3B). The same effect was observed in the plasma of PDT-treated animals. The antioxidant capacity of plasma was significantly reduced in these groups (P < 0.02) (Table 1).

**Antioxidant enzyme activities**

In tumours PDT produced strong induction of SOD. Twenty-four hours after the treatment, SOD achieved a high level of activity in both TMP (P < 0.02) and ZnTMP (P < 0.01) treated animals (Fig. 4A). At the same time, the tissue activity of CAT was roughly unchanged after PDT (Fig. 4B). On the contrary, GPx activity, which is also involved in hydrogen peroxide destruction, was significantly increased in TMP (P < 0.05) and ZnTMP (P < 0.01) treated animals. In this case ZnTMP seemed

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**Table 1. Oxidative stress markers in the plasma of tumour-bearing rats at 24 h after PDT treatment with TMP and ZnTMP**

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>TBARS nmol/ml</th>
<th>Protein carbonyl nmol/mg protein</th>
<th>Thiol groups μmol/ml</th>
<th>Antioxidant capacity μM eq. Trolox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>2.34 ± 0.30</td>
<td>0.61 ± 0.04</td>
<td>0.06 ± 0.01</td>
<td>22.4 ± 0.3</td>
</tr>
<tr>
<td>Irradiated</td>
<td>2.86 ± 0.21</td>
<td>0.57 ± 0.06</td>
<td>0.08 ± 0.08</td>
<td>22.9 ± 0.6</td>
</tr>
<tr>
<td>TMP</td>
<td>3.17 ± 0.17a</td>
<td>0.51 ± 0.05</td>
<td>0.10 ± 0.02</td>
<td>21.6 ± 0.1a</td>
</tr>
<tr>
<td>ZnTMP</td>
<td>3.17 ± 0.25a</td>
<td>0.65 ± 0.08</td>
<td>0.09 ± 0.05</td>
<td>21.3 ± 1.4</td>
</tr>
</tbody>
</table>

*a* Significant vs. controls (P < 0.05),

*b* Significant vs. controls (P < 0.02)
to produce a significantly higher activity than TMP (P < 0.02) (Fig. 4C).

The activities of erythrocyte antioxidant enzymes, excepting CAT, resembled those in the tumours: SOD and GPx were strongly increased in PDT-treated groups. CAT activity in lysates was significantly reduced in those groups (Table 2).

Discussion

The effects of PDT on tumour tissues and cells are the consequence of the reactions initiated after the absorption of light by a photosensitizer, which causes transition of PS from the ground state to the excited singlet state. It then either decays to the ground state or undergoes intersystem crossing to the triplet excited state. The excited sensitizer can react with surrounding oxygen molecules in two ways. In type I pathway, a direct reaction...
tion with substrates, which involves a hydrogen or electron transfer, determines the formation of radicals. These radicals may react with oxygen and produce free oxygen radicals, and a chain of reactions is initiated. In type II pathway, an energy transfer from the excited PS to molecular oxygen takes place, leading to singlet oxygen (°O₂) formation. Singlet oxygen is a reactive chemical species and interacts efficiently with susceptible substrates. These pathways of reactions occur simultaneously and competitively, the ratio between the two processes depending on the PS, substrate, and oxygen concentration. However, due to the high efficiency of singlet oxygen reaction with biomolecules, the type II pathway seems to play the most important role in cytotoxicity (De Rosa and Bentley, 2000; Jakus and Farkas, 2005). ROS produced in these chains of reactions causes major alterations in lipids, proteins, and DNA.

ROS measurement appears to be very important in evaluating their involvement in various diseases. Chemiluminescence represents production of light generated from chemical sources. It has been used for estimating ROS generation (Wardman, 2007). Luminol is a chemiluminescence enhancer functioning as bystander substrate for oxygenation. It can measure the levels of hydrogen peroxide, O₂°, ‘OH and other species, although it cannot distinguish these species from one another. Luminol chemiluminescence has been applied to determination of the global levels of ROS under different physiological and pathological conditions and has been useful for examining the kinetics and reaction mechanisms of oxygen radical processes.

The chemiluminescence results obtained in this in vivo experiment demonstrate a high level of ROS in tumour tissues at 18 h after PDT, which declines to a near control level by 24 h. This result shows that chain reactions induced by PDT and involving reactive species continue for a long time after irradiation.

The most frequently used indirect evidence for the presence and action of free radicals in the tissues is the level of lipid peroxides. Lipid peroxidation is a complex process produced by radicals or excited species that results in a variety of stable and toxic oxidized biocompounds presenting damaging effects on membranes. As a consequence of oxidative alteration, biomembranes lose their fluidity, their permeability to different ions increases and their membrane potential is reduced. Finally, the cell and organelle membranes are destroyed, which leads to cell destruction (Girotti, 2001).

The measurement of TBARS has been shown to be a sensitive index of lipid peroxidation. TBARS assay measures secondary products of lipid peroxidation such as 2,4-decadienols and, to a lesser extent, saturated aldehydes (Esterbauer and Chjeseman, 1994). ROS produced by PDT causes formation of conjugated diene, lipid hydroperoxides and finally aldehydes, which react with thiobarbituric acid.

We have chosen the thiobarbituric acid assay for lipid peroxidation in tumour tissue and blood in order to evaluate the local and systemic effects of PDT. As is seen in Fig. 2A and Table 1, PDT with both TMP and ZnTMP resulted in an increased level of TBARS as a measure of lipid peroxidation in tumour tissue as well as in blood plasma at 24 h after the treatment.

There are many in vivo and in vitro studies showing that PDT induces increased levels of lipid peroxides (Chaterjee et al., 1997; Streckyte et. al., 1999; Daicoviciu et al., 2008; Filip et al., 2008; Zaczkos, 2009). Moreover, it was shown in different cell lines that lipid peroxidation takes place within a few minutes after PDT. The levels of lipid peroxides fall to those in untreated controls at 18 h (Szacko et al., 2009).

In vitro studies with Hep2 cells treated with a series of 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrins and exposed to visible light have shown that the cytotoxic photodynamic effect of these porphyrins correlates with °O₂ production (Milanesio et al., 2001). These results suggested that the PS act via the type II mechanism of reaction.

Data obtained in our experimental model evidenced that in vivo administration of both TMP and ZnTMP are effective in producing high levels of reactive species, which may interact with lipids from cell membranes, leading to destructive effects on tumour tissues.

ROS have been implicated in oxidative modification of proteins. Oxidative changes in proteins represent one of the cellular effects of PDT. The main oxidative modifications that occur in proteins are carbonylation and thiol groups’ oxidation. As a result of changes produced by PDT in proteins, their activity is modified: reversible or irreversible alterations of their enzymatic functions or disturbance of protein-protein interactions take place.

In this experiment, significantly increased levels of protein carbonyls were recorded in tumour tissues as compared with controls and irradiated groups. ZnTMP produced a significantly higher concentration of oxidized protein compared to TMP. This difference might be accounted for by the higher production of °O₂ by ZnTMP. In vitro studies in Hep2 cells demonstrated that ZnTMP presents greater cytotoxicity than TMP. This effect was correlated with the higher level of °O₂ produced by the zinc derivative (Milanesio et al., 2001).

In vitro studies regarding PDT-induced protein carbonylation support the idea that oxidative damage to proteins is selective and that proteins with specific sensitivity to oxidation can regulate cellular signalling events including apoptosis (Sakharov et al., 2003; Magi et al., 2004; Tsaytler et al., 2008; Price et al., 2009).

Regarding thiol groups, it is well known that cysteine thiol groups of proteins are highly important for the function of metabolic enzymes and for signalling processes underlying responses to environmental factors. These groups are very vulnerable to oxidation. Thiol groups in cysteine reversibly change their redox state. As a consequence, alteration of structural, catalytic and regulatory functions takes place.

As in the case of protein carbonyls, the level of thiol groups in tumour tissues of rats were significantly altered after PDT with both porphyrins, demonstrating a
strong oxidative process at the level of proteins in the tumour. Similar results were obtained in rats treated with tetra-sulphophenyl porphyrin (TSPP) PDT. This treatment induced a progressive increase in protein carbonyls, beginning 3 h after the treatment and attaining the maximum level at 24 h after PDT. At the same time, the levels of thiol groups decreased to a minimum value at 24 h (Clichici et al., 2010).

Antioxidants act in mammals as defence mechanisms against the effects of excessive oxidation produced by ROS, contributing to maintenance of low, steady-state levels of these species of oxygen. The antioxidant capacity is determined by an interaction between individual components such as ascorbic acid, vitamin A, β-carotene, vitamin E, reduced glutathione, albumin and several antioxidative enzymes. The ABTS test used by us reflects the content of rapidly reacting antioxidants such as ascorbic acid, urate and some thiols.

The antioxidant capacity of tumour tissues shows strong impairment after 24 h from PDT with both porphyrins. ZnTMP seems to produce a more important effect on reducing the antioxidant levels than TMP. Plasma levels of protein carbonyls and of thiol groups, which were roughly unchanged, suggest that PDT had a limited effect on blood proteins. However, the plasma had a reduced antioxidant capacity. This result suggests that other plasma antioxidants than thiol groups are impaired by PDT.

Oxidative photodynamic damage to tumour cells leads to the activation of rescue antioxidative mechanisms involving antioxidant enzymes or mechanisms leading to cell death through apoptosis or necrosis.

The antioxidant enzymes are essential for the protection of cells from oxidative stress. There are at least three families of primary antioxidant enzymes in mammalian cells. Superoxide dismutases catalyse conversion of superoxide to hydrogen peroxide, which is removed by catalase or one of glutathione peroxidases. Changes in the activities of antioxidant enzymes during photosensitized irradiation may play an important role in modulation of cell viability.

The level of SOD in tumour cells is generally low. The present experimental results suggest a strong influence exerted by TMP and ZnTMP in combination with irradiation on the level of SOD activity. In tumour tissues of the treated animals, the SOD activity reached much higher levels than in the controls. In vitro PDT studies with different porphyrins in malignant and normal cells lines demonstrated an increase in SOD activity immediately after irradiation and a fall of activity at a several hour interval (Johnson and Pardini, 1998; Saczko et al., 2008). Moreover, inhibition of MnSOD, a mitochondrial form of the enzyme, in tumour cells showed an increase of the cytotoxic effect of PDT (Golab et al., 2003). These results suggest an important role for antioxidant enzyme MnSOD in cancer cell response to PDT. Hydrogen peroxide is the product of the dismutation reaction of superoxide radical. CAT is the primary route for its detoxification when hydrogen peroxide is present in high concentrations. On the other hand, GPx (Se) removes hydrogen peroxide when present at low steady-state concentrations. GPx also removes organic hydroperoxides.

In our experiment, unlike SOD, the CAT level in tumours was slightly reduced in irradiated and porphyrin-treated groups, while GPx showed a significant rise compared to controls.

After PDT the tumour cells could detoxify the formed H₂O₂ via endogenous CAT and glutathione redox cycle, which involves GPx. Our results are in agreement with those obtained in Ehrlich ascites carcinoma cells photosensitized with a haematoporphyrin derivative (Chekulayeva et al., 2004). On the other hand, the activity of CAT had a higher effect in Ehrlich ascites cells after PDT with haematoporphyrin. Moreover, a transitory increase of antioxidant enzymes SOD and CAT after PDT was observed in mammary carcinoma cells photosensitized with hypericin (Johnson and Pardini, 1998).

The results obtained in this experiment suggest an imbalance between the formation of hydrogen peroxide as a result of SOD activity and its removal by CAT and GPx at 24 h after therapy. There are evidences that hydrogen peroxide produced during irradiation of photosensitized cells contributes to PDT efficacy. The pro-apoptotic effect of H₂O₂ in PDT was demonstrated (Price et al., 2009). A high level of hydrogen peroxide in tumour tissues after PDT with TMP and ZnTMP might contribute to the treatment efficacy.

The activities of erythrocyte enzymes were significantly changed after PDT: while SOD and GPx levels increased, the CAT level decreased. This seems to be related to the damage to blood cell membranes and alteration of their protein structure due to the effect of ROS produced by PDT, as was evidenced in other investigations (El-Missiri and Abu-Seif, 2000).

Our study demonstrated that TMP and ZnTMP are efficient photosensitizers in vivo. PDT with TMP and ZnTMP caused ROS production in Walker 256 carcinoma tumour tissues. These agents induced oxidative changes of biomolecules and alteration of anti-oxidative systems. Tumour cell destruction could be the consequence of these alterations. Photodynamic therapy with these porphyrins in this experimental model also produced oxidative changes in the plasma and activated antioxidant enzymes in erythrocytes of the treated animals.

References


