Evaluation of the Photobiological Efficiency of $TPPS_4$ in Two Different Types of Mice Tumors

Ž. Lukšienė[1],
L. Rutkovskienė[1],
S. Jurkonienė[2],
G. Maksimov[2],
L. Griciūtė[1]

[1] *Lithuanian Oncology Center, Santariškių 1, LT-2007 Vilnius, Lithuania*
[2] *Institute of Botany, Žaliųjų ežerų 49, LT-2021 Vilnius, Lithuania*

A22 hepatoma and Ehrlich ascite carcinoma were used for evaluation of meso-tetra-(para-sulfo phenyl) porphyrin ($TPPS_4$) photobiological efficiency. According to the data obtained, $TPPS_4$ is a very effective photosensitizer in murine A22 hepatoma and absolutely ineffective in Ehrlich ascite carcinoma. Current data could be easily explained by the different accumulation potential of $TPPS_4$ in these types of tumor. A clear correlation was obtained between the photobiological efficiency and intracellular concentration of $TPPS_4$. Concerning the mechanism of tumor damage, it seems possible that $TPPS_4$ is mostly damaging the tumor vasculature and due to it could be effectively combined with other hydrophobic photosensitizers damaging tumor cells.

Key words: photodynamic therapy (PDT), photosensitizer, $TPPS_4$, Ehrlich ascites carcinoma, A22 hepatoma

INTRODUCTION

Photodynamic therapy (PDT) has been designated as a "promising new modality" in the treatment of cancer since the early 1980s. The point is that interaction of absolutely non-toxic visible light with photosensitizer accumulated in the tumor (in the presence of oxygen) could produce more or less selective tumor destruction without damage of surrounding normal tissue (1).

One of the most widely accepted photosensitizers – photofrin (PII) – has been approved by US FDA and the authorities of Canada, Japan, The Nedherlands, France and Germany for several clinical applications. Unfortunately, PII has "enough" side effects and disadvantages: chemically not pure, low absorbance in the red region of visible light (where the highest light penetration into tissues occurs) and long (4–6 weeks) skin phototoxicity (edema and erithema) (1).

Thus, new second generation photosensitizers with different photophysical, photochemical and photobiological properties were developed. The meso-tetra-(para-sulfo phenyl) porphyrin ($TPPS_4$) is one of them. According to (1), due to high hydrophility this sensitizer is taken up by endocytosis and finally accumulated in lysosomes of malignant cells. Moreover, its ability to generate singlet oxygen, the main cell killer, is very high (2). Concerning the $TPPS_4$ photobiological activity, experimental data are controversial: some authors indicate complete remission of neoplastic lesions in human skin after topical application of this sensitizer and light, while others note a rather low photobiological activity in murine tumor model (3, 4).

This controversy is perhaps caused by an incomplete understanding of sensitizer accumulation, a process that depends mostly not on just its physico-chemical properties, but also on the specifity of tumor, vascularization degree and cell membrane histoarchitecture. In order to understand more deeply the $TPPS_4$ phototoxicity mechanisms and to confirm our hypothesis, we decided to make a comparative analysis of $TPPS_4$ accumulation, phototoxicity and the ways of tumor destruction in two different murine tumor models: Ehrlich ascites carcinoma and solid A22 hepatoma.

MATERIALS AND METHODS

Chemicals and photosensitizers. Meso-tetra-(para-sulfo phenyl) porphyrin ($TPPS_4$) (a gift from K. Berg, Norway) was prepared in sterile physiological saline (0.9% NaCl) (2.5×10^{-3} M). Solution was sterilized and stored below 10 °C in the dark. The chemical structure of the photosensitizer is presented in Fig. 1.

Object and experimental aparatus. The experiments were carried out using the BALB/c mice strain. Ehrlich ascites carcinoma was transplanted into female mice aged 6–7 weeks and weighing approximately 21 g. The implantation procedure is

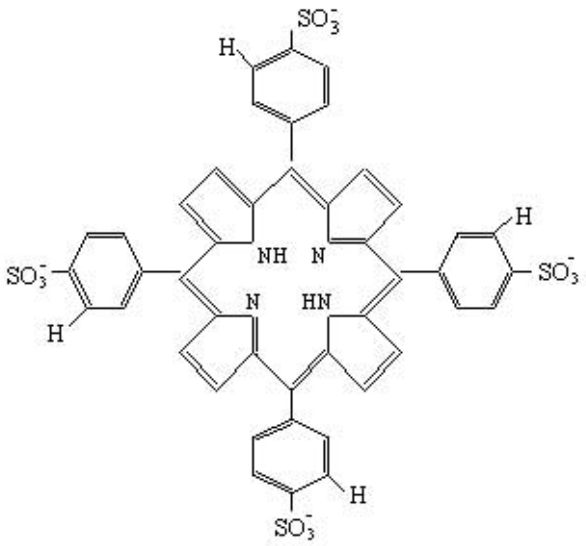

Fig. 1. Chemical structure of the $TPPS_4$

summarized as follows: a tumor is dissected from a donor mouse and E. ascites tumor cells (0.3 ml) are inoculated intraperitoneally (i. p.) using a 25 G needle to healthy mice.

On the 7th day after tumor inoculation, in its exponential growth phase $TPPS_4$ was injected i. p. 40 mg/kg (body weight) as an optimal concentration for this type of tumor which had been evaluated before (5). After 3 h of incubation Ehrlich ascites tumor cells were excluded from the intraperitoneum and prepared *ex vivo* in the dark as a homogeneous cell suspension with the optical density at 590 nm OD = 0.6 (3.7×10^6 cells/ml). This incubation time was picked up as optimal for sensitizer delivering in this type of tumor (6). Irradiation of cells was performed in 2 mm cuvettes. After treatment, 0.2 ml of irradiated cell suspension (0.75×10^6 cells) was inoculated in healthy mice i. p. and tumor growth was measured for 15 and more days. Every group consisted of 8 mice. The control group was inoculated with an untreated EAT cell suspension. All experiments were done in the dark and repeated 3 times.

The procedure of hepatoma A22 implantation into CBA x C_{57} black mice could be summarized as follows. Tumor mass was separated from connective tissues, rinsed in physiological saline (0.9% NaCl) and cut into small pieces, passed through a 26-gauge needle and then implanted into the right leg of the recipient mouse (0.3 ml tumor cell suspension). The tumors grew to a volume of about 100–150 mm^3 within 6–7 days, and after the mice were used for experiments. The $TPPS_4$ solution was injected i. p. into mice and following 24 h topical irradiation of tumor was performed (7). The control mice group with A22 hepatoma was not treated at all. Every group consisted of 6 mice, all experiments were repeated 3 times.

The mice were under general anesthesia (ketamine hydrochloride, i.p.) during all experiments.

Light sources. The light source used for irradiation of Ehrlich ascites carcinoma cell suspension consisted of tungsten lamp (500 W), optical system for light focusing, and optical filter for UV and infrared light elimination (370 nm < λ < 680 nm). Light intensity at the position of the cells was 50 mW/cm^2. The irradiation time was 90 s.

A non-laser light source for irradiation of solid tumors was constructed at the Laser Center of Vilnius University. The wavelength of excitation for $TPPS_4$ was 630 nm, bandwith 50 nm. The power of produced radiation was 120 mW and the intensity of light in the focus (assuming the diameter of the spot as 10 mm) exceded 90 mW/cm^2. Irradiation time to reach total tumor necrosis was 35 min (7).

Tumor growth determination. Relative Ehrlich ascites tumor growth was measured every day up to day 15 of its growth according to the equation:

$$S = (S_1 - S_0)/S_0,$$

where S_1 is the final weight of mouse with tumor, S_0 is the initial weight of intact mouse, and S stands for relative tumor growth.

Moreover, Ehrlich ascites tumor growth was measured by two other ways:

1) absolute tumor volume growth during 15 days;

2) tumor cell number during 15 days.

The correlation between absolute tumor weight and relative tumor growth was found very strong (r = 0.98). In order to simplify the experimental protocol, we usually measured only relative tumor growth (8).

The volume of A22 hepatoma was measured *in vivo* and calculated:

$$V = 1/2(4\pi/3) \times (l/2) \times (w/2) \times h,$$

where l is the longer perpendicular axis, w is the shorter perpendicular axis, and h is the height of ellipsoidal tumor (9).

Measurements of intracellular concentration of photosensitizer. Ehrlich ascite was collected from the mice 3 h after treatment with photosensitizer. Tumor cells were suspended in phosphate-buffer solution (PBS) to an optical density OD = 0.6. The fluorescence of the suspension was measured with a СФР – 1 spectrofluorimeter (Moscow, Russia) at λ = 600–680 nm (10). An EAT suspension treated in the same manner without photosensitizer was

taken as control. Standard curves were produced by adding a known amount of the photosensitizer.

A22 hepatoma tumor was separated from the mice 24 h after treatment with photosensitizer, and tumor cells were suspended in PBS to an optical density OD = 0.8.

Fluorescence microscopy. Fluorescence measurements were performed with a NICON Eclipse-E-400 fluorescence microscope (Japan). To detect $TPPS_4$ localization in the cell, a light excitation band λ_{exc} = 450–490 nm was used.

Protein quantitation. The quantitation of protein was determined by the Bradford method (11) in cell suspension.

Pathohistological examination. The tumor tissue was fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Statistical evaluations. All experiments were repeated at least three times. Averaged values and standard deviations were calculated.

RESULTS AND DISCUSSION

It is well established that for experiments *in vitro* $TPPS_4$ localizes mainly within lysosomes (1, 13). Under the conditions of the present study the fluorescence of $TPPS_4$ in EAT cells was to a large degree concentrated in spots. These spots or granules were distributed all over the cells except for the nuclear region. In all cases an additional diffuse fluorescence was seen all over the cells. For better understanding the fluorescence from lysosomes we used AO, which when localized in these organelles emitted orange and red (>600 nm) fluorescence.

To evaluate the phototoxicity of $TPPS_4$ in Ehrlich ascites carcinoma, we observed tumor growth after PDT for 15 days and compared it with a control (not treated) tumor growth (Fig. 2).

The data obtained show that tumor growth in $TPPS_4$ – PDT treated mice did not differ from tumor growth in control. Does the photobiological activity of $TPPS_4$ depend on the histological type of tumor? To answer this question we performed a $TPPS_4$–PDT treatment of mice bearing hepatoma A22. Figure 3 shows that during 10 days after PDT treatment in treated mice the tumor growth was significantly suppressed. $TPPS_4$ seems to be rather effective in this type of tumor.

What is the reason for such remarkable differences in $TPPS_4$ phototoxicity in two different tumor types? It is obvious that the efficiency of photodynamic therapy is a rather complicated function with a lot of determinants (12). As mentioned above, the photophysical properties of sensitizer, such as aggregation, photobleaching, singlet oxygen generation, etc. can modulate the level of photodamage. Of course, the efficiency of the photodynamic process strongly depends on the pattern of dye localization in cells and subcellular compartments (13).

Moreover, it has been suggested that the sensitizer accumulation potential in tumor may be related to its binding to plasma protein. For instance, human serum albumin (HSA) is “responsible” for the accumulation of sensitizer in tumor vasculature, while low density lipoproteins (LDL) and high density lipoproteins (HDL) manage its accumulation in tumor cells. Hydrophilic photosensitizers mostly bind to HSA, hydrophobic to LDL and HDL (14). As evaluated in (15), $TPPS_4$ binding ability to HSA seems far higher than PII, hematoporphyrin dimethylether (HPde) or chlorin e_6 (Cl e_6).

Thus, it seems evident that intracellular accumulation of sensitizer is one of the most important factors in determinig the efficiency of PDT treatment. Thus, the $TPPS_4$ accumulation potential in the cells of both type tumors was tested. According to fluorimetric data of intracellular $TPPS_4$ content, the accumulation potential of it in A22 hepatoma is

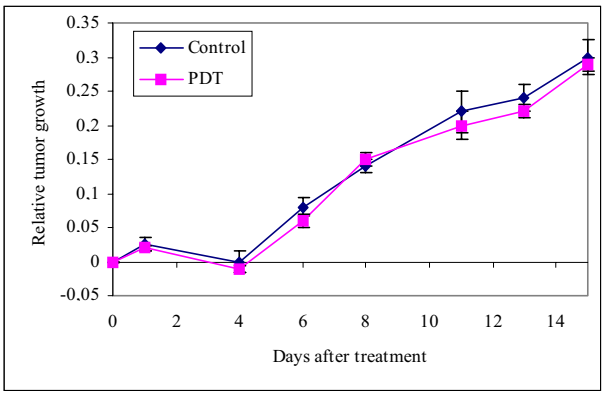

Fig. 2. Inhibition of Ehrlich ascites carcinoma growth after $TPPS_4$-based photodynamic therapy

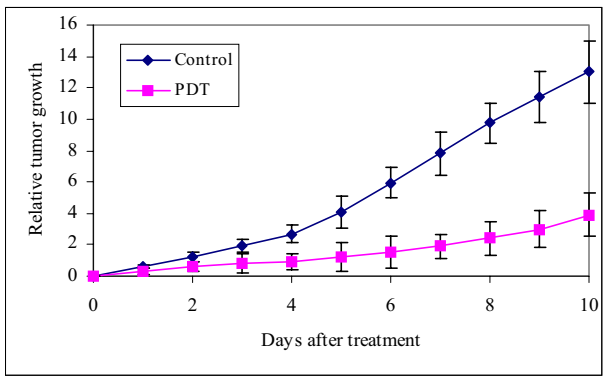

Fig. 3. Inhibition of hepatoma A22 growth after $TPPS_4$-based photodynamic therapy

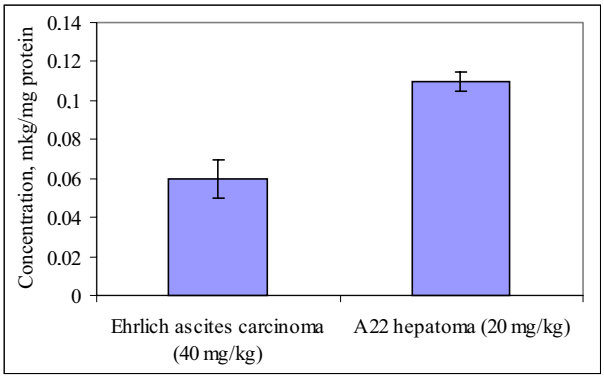

Fig. 4. $TPPS_4$ intracellular concentration in mice Ehrlich ascites carcinoma and A22 hepatoma

about two times higher than in Ehrlich ascites carcinoma (Fig. 4). We must note that in the case of Ehrlich carcinoma, due to ascite tumor, the i. p. used $TPPS_4$ concentration was 40 mg/kg, and in the case of A22 hepatoma only 20 mg/kg. It means that $TPPS_4$, under the best experimental conditions, accumulates in Ehrlich ascites carcinoma very slowly.

Concerning localization in solid tumor tissue, there is an opinion that $TPPS_4$ distributes preferentially in tumor stroma (16). In general, water-soluble sensitizers kill neoplastic cells indirectly by damaging blood vessels and interrupting the supply of oxygen and other essential nutrients (17). Our observations of $TPPS_4$ fluorescence in A22 hepatoma tissue by fluorescence microscope confirmed the idea that this drug does not accumulate significantly in this tumor. Figures 5 and 6 show the histological pictures of A22 hepatoma, non-treated and treated by with $TPPS_4$–PDT. According to our pathohistological evaluations of tumor slices, it is possible that $TPPS_4$-based PDT damages first of all the vasculature of tumor. Presumably a secondary tumor necrosis was observed 24 h following treatment.

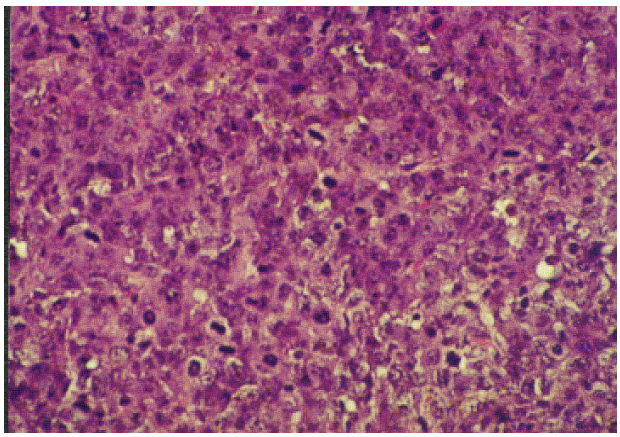

Fig. 5. Histological picture of growing transplantable hepatoma (control group). Tumor consists of polymorphous, mostly roundshape cells with hyperchromatic nuclei. There are numerous mitoses, some of them atypical, hematoxylin–eosin, 10 × 20

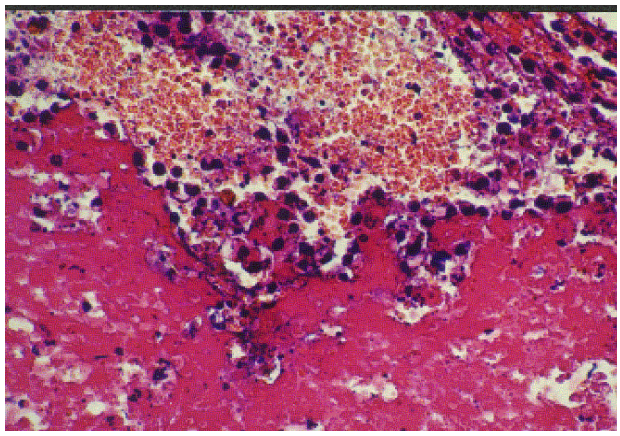

Fig. 6. Histological picture of hepatoma 24 h after treatment. Almost total necrosis of tumor. There are no growing cells. There are areas of shadows of necrotising cells. Wide hematomas result from the damaged blood vessels; hematoxylin–eosin, 10 × 20

To sum it up, it seems evident that $TPPS_4$ as a hydrophilic photosensitizer is rather effective in treating solid tumors. It is obvious that $TPPS_4$ is well accumulating in tumor tissue and localized mostly in tumor stroma. After PDT treatment, a remarkable damage of tumor vasculature was observed. Under hypoxic conditions, secondary necrosis of tumor tissue was evaluated and a significant inhibition of tumor growth was detected. In the case of ascite tumor (EAT), the accumulation potential of $TPPS_4$ was very low and no tumor growth inhibition was observed, either. It means that in some special cases $TPPS_4$ can be a very effective hydrophilic photosensitizer directly damaging the vasculature of tumor and to enhance the PDT efficiency it could be combined with hydrophobic photosensitizers damaging directly tumor cells.

ACKNOWLEDGEMENTS

This study was financially supported by the Lithuanian State Science and Studies Foundation (Grant No. K-024).

Received 8 September 2001
Accepted 11 November 2001

References

1. Berg K, Western A, Bommer JC, Moan J. Intracellular localization of sulphonated meso-tetraphenylporphines in a human carcinoma cell line. Photochem Photobiol 1990; 52: 481–7.

2. Rodgers MAJ. The photoproperties of porphyrins in model biological environments. In: Jori G, Perria C, eds. Photodynamic Therapy of Tumours and Other Diseases, Liberia Progetto Editore, Padua, 1985: 21–35.
3. Evensen JF, Moan J. A test of different photosensitizers for photodynamic treatment of cancer in murine tumour model. Photochem Photobiol 1987; 46: 859–65.
4. Secchini V, Melloni E, Marchesini R, FabrizioT, Cascinelli N, Santoro O et al. Topical administration of $TPPS_4$ and red light irradiation for the treatment of superficial neoplastic lesions. Tumori 1987; 73: 19–23.
5. Lukšienė Z, Juodka B. Porphyrins as radiosensitizers in ^{60}Co irradiated Ehrlich ascite tumor cells: possibility to combine ^{60}Co irradiation with PDT. In: Dougherty T, ed. Proc. Optical Methods for Tumor Treatment and Detection: Mechanisms and Technique in Photodynamic Treatment. Washington, 1993; 1881: 67–75.
6. Lukšienė Z, Atkočius V, Juodka B. Radiosensitization by porphyrins in ^{60}Co irradiated cells of different nature. SPIE Proc Photodynamic Therapy of Cancer, 1994; 2078: 447–9.
7. Lukšienė Z, Rutkovskienė L, Griciūtė L, Vaicaitis V, Sirutkaitis V. A new non-coherent light source for photodynamic treatment of cancer. Acta Medica Lithuanica 2002; 1, in press.
8. Lukšienė Z, Kalvelytė A, Supino R. On the combination of photodynamic therapy with ionizing radiation. J Photochem Photobiol 1999; 52: 35–42.
9. Amano T, Prout GR, Lin Chi-Wei. Intratumoral injection as a more effective means of porphyrin administration for photodynamic therapy. J Urology 1998; 139: 392–5.
10. Ivkova MN, Ivkov VG, Peechatnikov VA, Pletnev VV, Bukolova-Orlova TG, Aphanaiev VN. Distribution of potential-sensitive probe dis-C_3-(5) in membrane suspension. Gen Physiol Biophys 1982; 1: 209–19.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein–dye binding. Analyt Biochem 1976; 72: 249–54.
12. Moan J et al. Intracellular localization of photosensitizers. In: Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use. Wiley, Chichester, 1999; 95–107.
13. Lukšienė Z, Putaitė D, Juodka B. Effect of photosensitizers and light on biomolecules and cells. Investigation of some physico-chemical properties of porphyrins, which determine the efficiency of *Saccharomyces cerevisiae* photosensitization *in vitro*. Exp Biol 1992; 2: 36–43.
14. Schuitmaker JJ, Baas P, van-Leengoed HL, van-der-Meulen FW, Star WM, van-Zandwijk N. Photodynamic therapy: a promising new modality for the treatment of cancer. J Photochem Photobiol 1996; 34: 3–12.
15. Jasaitis A, Streckytė G, Rotomskis R. Binding of porphyrin-type sensitizers to human serum albumin: spectroscopic investigation. Biologija 1997; 2: 16–23.
16. Henderson BW, Dougherty TY. How does photodynamic therapy work? Photochem Photobiol 1992; 55: 147–57.
17. Rosenthal J. Phthalocyanines as photodynamic sensitizers. Photochem Photobiol 1991; 53: 859–70.

Ž. Lukšienė, L. Rutkovskienė, S. Jurkonienė, G. Maksimovas, L. Griciūtė

$TPPS_4$ EFEKTYVUMO ĮVERTINIMAS DVIEJŲ SKIRTINGŲ PELIŲ NAVIKŲ MODELIUOSE

S a n t r a u k a

Buvo tirtas sensibilizatoriaus $TPPS_4$ fotobiologinis aktyvumas dviejuose skirtinguose pelių navikų tipuose: Ehrlicho ascitinėje karcinomoje ir A22 hepatomoje. Eksperimentai parodė, kad $TPPS_4$ yra efektyvus solidiniame A22 hepatomos naviko atveju ir visiškai neefektyvus esant Ehrlicho ascitinei karcinomai. Tai galima paaiškinti skirtinga $TPPS_4$ akumuliacija šiuose skirtingos histogenezės navikuose. Sensibilizatoriaus fotobiologinis aktyvumas tiesiogiai koreliuoja su jo viduląsteline koncentracija.

Raktažodžiai: $TPPS_4$, Ehricho ascitinė karcinoma, A22 hepatoma