THE EFFECT OF THE PHOTODYNAMIC THERAPY BY USE OF THE KRYPTONLASER AND META-OH-PARA/CH₃/₃-TETRAPHENYLPORPHYRIN ON CELLS OF TRANSPLANTABLE MAMMARY CANCER

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Abstract - The results of the photodynamic therapy by use of krypton-laser and meta-OH-para/CH₃/₃-tetraphenylporphyrin on cells of transplantable mammary cancer are presented. Irradiation time dependent decrease of the number of cells and superoxide dismutase-activity in experimental group was confirmed.

1. Introduction.

Photodynamic therapy /PDT/ is well known method of malignant tumours treatment /1,2/, in which singlet oxygen, that subsequently impairs cellular organelles, is generated after some physico-chemical reactions /3/.

Superoxide dismutase is supposed to be one of the cellular enzymes, that "neutralizes" free radicals, e.g. singlet oxygen, in intracellular space.

In the last few years among many photosensitizers examined, the meso-tetra-hydroxyphenyl-porphyrins /THPP/ showed according to observations of some investigators very promising effect in treatment of tumours /4,5,6/.

The meta-OH-para/CH₃/₃-tetraphenylporphyrin, TPP, shows very similar structure to one of above mentioned compounds, i.e. meta-THPP.

Therefore we decided to examine the influence of TPP on viable cells of mammary cancer originating from Wistar rats.

This photosensitizer has not been yet involved to PDT studies. The waveband selected for use was 647.1 nm as the well tissue-penetrating wavelength adapted to the absorption spectrum of the porphyrin and producing effective cell or tumour damage /7/.

2. Materials and methods.

- a. porphyrin /see fig. 1/, meta-OH-para/CH₃/₃-tetraphenylporphyrin was prepared at Institute of Chemistry, University of Wrocław. The purity of this compound was confirmed by using electronic spectroscopy and proton magnetic resonance.

META-(OH)-PARA-(CH₃)₃-TPP

Fig. 1

Fig. 2. The correlation between time of irradiation and number of cells exposed to therapy

- b. cells, tumour cells derived from passages on Wistar rats were incubated in culture medium for 3 hours at 37°C with sensitizer. Histologically there were carcinoma solidum mammae cells.
- c. light source - the illumination of samples was performed by a krypton-laser ILK 120 /Carl Zeiss Jena, FRG/, the 647.1 nm line was used. The power of this irradiation was set at 240mW. The diameter of the laser beam was about 3 mm.

Tumour cells, in amount of 5×10^6 , in sterile conditions were exposed to action of TPP solution, i.e. 1mg of the sensitizer in 0,5 ml chloroform, and after 3 hours were irradiated with red light for 20, 40, and 60 seconds, it was experimental group. At the dose 1 mg/0,5 ml the cytotoxic effect was minimal and ignored. The cells in the same amount were exposed entirely to the action of the sensitizer alone / 1 mg/0,5 ml / - the control group I. The cells in the same amount were left without any further treatment - control group II. Finally, the same amount of cells were exposed to the action of laser alone - control group III.

The activity of superoxide dismutase /SOD/ was measured according to procedure described by Kurzawski / unpublished data, 1991 /. The SOD - activities were measured after 20, 40, and 60 seconds of irradiation in all studied, i.e. four, groups.

Results and discussion.

The results of examination are presented on table 1./see end of text. The extension of irradiation time causes the distinct decrease of the cells number in presence of the sensitizer. The most significant decrease was observed in the first 20 seconds. In the next 40 second / i.e. after 40 and 60 seconds of irradiation/ this process seems to be decelerated. In controls we have observed a slight decrease of cells number after laser / with no sensitizer / application.

The activity of SOD decreased in the greatest extent during the first 20 seconds of irradiation in experimental group. There was slighter decrease observed in the following 20 and 40 seconds. These results are compatible with the cytotoxic effect of therapy on cells in the same intervals of irradiation. In control groups we have observed only trivial differences independent of irradiation time.

The determination of SOD- activity, one of enzymes delaying singlet oxygen generation / Kwiatkowska, personal communication /, may be useful in monitoring the efficacy of the phototherapy. Briefly, the lower activities of superoxide dismutase mean the higher probability of tumour-cell destruction.

The most interesting fact in our study is, that the continuation of irradiation did not cause the rectilinear decrease of the number of cells. This decrease conditioned by red light and porphyrin therapy may be compared with hyperbole./ see fig. 2 under the figure 1/. Probably the hundred-per-cent cytotoxic effect, i.e. death of the total cell population, could have been obtained after the very long time of irradiation or after application of high-power density laser equipment. Unfortunately, in the latter the thermal effect may occur

The mechanisms of the phototoxic effect of porphyrins used in PDT and inactivation of various enzymes are poorly understood /8/. Sites of photodamage on cellular level are rather numerous, e.g. nucleus and cell membrane /9/ or mitochondria /10/. Our present results suggesting the possible role of enzymatic alterations in mitochondria or cytosol on the induction of photodamage.

Table 1. Results of the treatment. Irradiation time-dependent decrease of the number of cells and SOD-activity in experimental group in comparison with control groups

Time (sec.)	Experimental (number of cells)	Control 1 (number of cells)	Control 2 (number of cells)	Control 3 (number of cells)
20	$5 \cdot 10^5$	$5 \cdot 10^6$	$5 \cdot 10^6$	$4,2 \cdot 10^6$
40	$3,7 \cdot 10^4$	$5 \cdot 10^6$	$5 \cdot 10^6$	$4,2 \cdot 10^6$
60	$2,5 \cdot 10^4$	$5 \cdot 10^6$	$5 \cdot 10^6$	$4,2 \cdot 10^6$
	SOD-activity	SOD-activity	SOD-activity	SOD-activity
20	70 %	83,5 %	83 %	83 %
40	66 %	82 %	81 %	82 %
60	64,5 %	82 %	80 %	82,5 %

SOD - superoxide dysmutase

References:

1. Bergh H.v.d., Light and porphyrins in cancer therapy, *Chemistry in Britain*, May, 1986, 430.
2. Berns M.W., Wile A.G., Hematoporphyrin phototherapy of cancer, *Radiotherapy and oncology*, 7, 1986, 233.
3. Keene J.P., Kessel D., Land E.J., Redmond R.W., Truscott T.G., Direct detection of singlet oxygen sensitized by hematoporphyrin and related compounds, *Photochem. Photobiol.*, 43, 1986, 117.
4. Berenbaum M.C., Akande S.L., Bonnett R., Kaur H., Ioannou S., White R.D., Winfield U.J., Meso-tetra/hydroxyphenyl/ porphyrins, a new class of potent tumour photosensitizers with favourable selectivity, *Br.J.Cancer*, 54, 1986, 717.
5. Bonnett R., Ioannou S., White R.D., Winfield U.J., Berenbaum M.C., Meso-tetra/hydroxyphenyl/porphyrins as tumour photosensitizers: chemical and photochemical aspects, *Photobiochem. Photobiophys. Suppl.*, 1987, 45.
6. Bonnett R., McGarvey D.J., Harriman A., Land E.J., Truscott T.G., Winfield U.J., Photophysical properties of meso-tetraphenylporphyrin and some meso-tetra/hydroxyphenyl/ porphyrins, *Photochem. Photobiol.* 48, 1988, 271.
7. Gemert J.C., Berenbaum M.C., Gijssbers G.H.M., Wavelength and light-dose dependence in tumour phototherapy with hematoporphyrin derivative, *Br.J.Cancer*, 52, 1985, 43.
8. Maziere J.C., Santus R., Morliere P., Reyftmann J.-P., Candide C., Mora L., Salmon S., Maziere C., Gatt S., Dubertret L., Cellular uptake and photosensitizing properties of anticancer porphyrins in cell membranes and low and high density lipoproteins, *J.Photochem. Photobiol., B: Biology*, 6, 1990, 61.
9. Santus R., Reyftmann J.-P., Photosensitization of membrane components, *Biochimie*, 68, 1986, 843.
10. Salet C., Moreno G., Photosensitization of mitochondria. Molecular and cellular aspects, *J.Photochem. Photobiol., B: Biology*, 5, 1990, 133