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# Activation of porphyrins with waves of different spectra: new possibilities in the treatment of malignant tumors

**Laima Bloznelytė-Plėšnienė[1*],**

**Laimutė Rutkovskienė[1,2],**

**Albinas Plėšnys[3]**

[1] *Institute of Oncology, Vilnius University, Santariškių 1, LT-08406 Vilnius, Lithuania*

[2] *Department of Biochemistry and Biophysics, Vilnius University, Vilnius, Lithuania*

[3] *Department of Philosophy, Vilnius University, Vilnius, Lithuania*

We hypothesized that porphyrins in cancerous tissues can be activated not only by visible or ultraviolet light. Suitable wavelengths of ionizing radiation rays of a certain power are themselves capable of activating porphyrins. We have carried out experiments with hematoporphyrin derivative (HpD) solutions by activating them with visible light and γ rays from radioactive $^{60}$Co. On obtaining positive results with HpD solutions, *in vivo* experiments with C6 rat glioma were performed.

In experiments with HpD solutions, when HpD concentration was adequate, during illumination of such solution with the blue light a crimson fluorescence was established. However, it disappeared when the solution underwent irradiation with γ rays (2 Gy). But if the solution was covered with soda-lime glass and exposed to irradiation with γ rays (2 Gy), the crimson fluorescence under illumination with the blue light was fixed again.

Referring to our results, we may regard soda-lime glass as a filter which can eliminate the essential wavelength (or wavelengths) of the γ-ray spectrum capable of HpD activation. Detection of these wavelengths would enable researchers to find other ionizing radiation sources capable of activating some porphyrins more effectively.

**Key words**: sensitized tumor treatment, porphyrins, soda-lime glass

## INTRODUCTION

The possibilities of the methodology currently used in oncology are rather limited. Therefore, there is a call for a constant search for new, perspective treatment methods. One of such methods is the sensitized tumor therapy (STT) based on a quite selective porphyrin accumulation in tumorous and in some other rapidly proliferating tissues. STT is a method of treatment where accumulated endogenic and exogenous porphyrins in cancerous tissues are activated by a suitable wavelength of low intensity electromagnetic vibrations [1–3]. After absorbing the light quantum of a particular length, porphyrins resolve and destroy the tumorous tissue that had accumulated it. The most common method of STT is photosensitized tumor therapy, also known as photodynamic therapy (PDT) [4, 5]. Unfortunately, the visible light used during PDT penetrates into tissues only several centimeters deep. This narrows the application of STT. Therefore new, more penetrative ways of activating porphyrin are being sought. However, most of such currently carried out studies are directed towards looking for light sources which would radiate a longer (more penetrative) light in the visible part of the spectrum.

We have come up with the idea that porphyrins in cancerous tissues can be activated not only by the visible or ultraviolet light. Suitable wavelengths of different spectra are themselves capable of activating porphyrins as well. We suggested a new STT methodology which was called gammadynamic treatment (GDT) and which could expand STT possibilities in oncology [6]. The main idea of this STT methodology is that suitable wavelengths of ionizing radiation rays of a certain power are themselves capable of activating porphyrins. Thus, to activate an accumulated sensitizer in a tumor, a small amount of suitable gamma rays should be efficient. To test the presumption, we carried out experiments on mice and rats which were vaccinated with different types of inoculated animal tumor strains. The results of our experimental investigations indicate that after GDT some types of malignant tumors completely disappear in mice and rats, depending on the histological type of the tumor. The optimal single dose of gamma rays also depends on the histological type of the tumor and varies from 1.5 to 2 Gy. The optimal total dose of gamma rays varies from 4.5 to 6 Gy [7, 8].

* Corresponding author. E-mail: pdt@mail.lt

## MATERIALS AND METHODS

**Experiments with hematoporphyrin derivative (HpD) solutions exclusively.** The investigations were performed with hematoporphyrin derivative (Photogemum, Photogem Company, Russia) solutions of different concentrations. The concentration of HpD solutions varied from 0.0001 to 10 mg/ml (HpD was dissolved in 0.9% sol. NaCl). During illumination of the HpD solution with the blue light ($\lambda = 405$ nm), a slight red fluorescence was established.

**Cell culture.** C6 rat glioma cells were passaged in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 2 mM glutamine and penicillin–streptomycin. The cultures were kept at 37 °C in a humidified atmosphere containing 95% of air and 5% of $CO_2$. After trypsinization the cells were adjusted to an appropriate concentration in 0.9% NaCl for intracerebral or subcutaneous inoculation.

**Implantation of C6 glioma cells**. Wistar male rats (the facility of Immunology Institute) at 10–12 weeks of age and of 300–400 g body weight were used throughout the study. The animals received care in accordance with the guidelines established by the Lithuanian Animal Care Committee which approved the study.

For intracerebral (i.c.) inoculation, the rats were anesthetized with a mixture of ketamine hydrochloride (Bioketan) and xylazine by intraperitoneal injection. The head was sterilized with 5% iodine and a burr hole (2 mm diameter) was made with a hand drill in the right cranial bone. Saline solution (50 µl, containing $5\times10^5$ cells) was stereotactically implanted at the depth of 4 mm from the surface. For subcutaneous (s.c.) inoculation, the suspension of $1\times10^7$ cells in 500 µl of saline solution was inoculated into the left thigh of male Wistar rats.

**Experimental design and irradiation**. On the 11th day after i.c. implantation and on the 10th after s.c. inoculation the rats were divided into the investigational and control groups. The investigational group of rats underwent GDT: the HpD solution was injected into each rat-tail vein at a dose of 5 mg/kg body weight. 24, 48 and 72 h after the injection of the sensitizer the tumors were irradiated with gamma rays from radioactive $^{60}$Co (2 Gy as a single dose, the full dose of the course 6 Gy). The second investigation group of rats underwent GDT, but at time of irradiation these tumors were covered with 5 mm thick soda-lime glass. In soda-lime glass structure, sodium ions ($Na^+$) and calcium ions ($Ca^{2+}$) are inserted into the structure of the silicate ion so that the tetrahedron made of silicon and oxygen atoms is stretched.

**Evaluation of tumor progression**. When tumors were inoculated stereotactilly in rat brain, the survival time of the rats in each group was estimated and compared. The rats were examined for the behavioral and neurologic signs of tumor growth as well. When the tumors were inoculated s.c., the size of the tumor was measured with slide calipers every 2–3 days. The volume of the tumor was defined as $V=1/2 \times (4\pi/3) \times (a/2) \times (b/2) \times c$ (a: length, b: width, c: height of the tumor). Relative tumor growth was calculated according to the equation: $S = (S_n–S_0)/S_0$, where $S_n$ is the final and $S_0$ the initial volume of tumor. On day 16 following inoculation, a slow spontaneous regression of the tumors was detected; it corresponded to the data of other authors [9, 10].

**Statistical analysis**. For data analysis of the experiment with i.c. C6 glioma, the program package SAS (Statistical Analysis Systems) was made use of. For the experiment with s.c. C6 glioma, statistical comparisons were made by One-Way Analysis of Variance using computerized software (SigmaStat, Version 3.0). All pairwise multiple comparison procedures were performed following the Student–Newman–Keuls method. The significance was defined as a minimum of 0.05 for each comparison.

## RESULTS AND DISCUSSION

We have carried out a large number of experiments with HpD solutions and assessed the changes that appeared in HpD solutions after activating them with electromagnetic vibrations of different spectra: the visible light and ionizing radiation. We compared the differences and similarities of those changes: in some experiments the spectroscopic analysis of the samples was used, and in the others the intensity of the fluorescence of HpD solutions was tested.

When performing experiments with hematoporphyrin derivative solutions, the most interesting result was noted when the concentration of the HpD solutions varied within 0.02–0.175 mg/ml. During the illumination of a drop of such HpD solution with the blue light, a slight crimson fluorescence was established. However, the crimson fluorescence immediately disappeared when the solution underwent irradiation with gamma rays from radioactive $^{60}$Co (2 Gy as single dose). Moreover, if the drop of HpD solution (0.005 ml) was covered with soda-lime glass and then was irradiated with gamma rays (2 Gy) from radioactive $^{60}$Co, the red fluorescence was present under illumination of such solution with the blue light. It is essential to note that the other types of glass exerted no change in the fluorescence.

The positive results with HpD solutions encouraged us to search for other ionizing radiation sources except radioactive $^{60}$Co, which might be capable of activating some porphyrins more effectively *in vivo* with rats to whom glioma C6 had been inoculated.

Fifty rats were divided into two investigational groups (HpD+Gy and HpD+Gy+glass) and three control groups ($n = 6–9$). Rats with C6 glioma without further treatment comprised the control group. The HpD control group received only HpD (5 mg/kg body weight) injection without any other treatment. The Gy control group consisted of rats which underwent the same treatment

as the rats of the first investigational group, except HpD injection.

When the tumors were inoculated stereotactilly in rat brain, differences of the rat behavior were noted in both investigational and in all control groups. The rats without any treatment and the ones that underwent only HpD injection showed neurologic signs of tumor growth on the 12th day after tumor inoculation. The signs included decreased alertness, passivity, poor grooming, irritability and neurologic deficits such as gait disturbance. These signs were indicative of the increasing intracranial pressure as the tumor expanded. The progression of these signs was noted in both control groups during all experimental time. In the investigational group that underwent GDT without any modification, neurologic signs of tumor growth appeared on the 12th day after tumor inoculation as well. However, the disappearance of these signs was noted immediately after the gammadynamic treatment. In some of the treated rats the recurrence of neurological signs was found on the 20th – 22nd day of treatment. As regards the Gy control group, the neurologic signs of tumor growth appeared on the tenth day after tumor inoculation and a slow progression of these signs was fixed during all experimental time. An analogous situation was found in the HpD+Gy+glass group.

The survival time of rats in the control and investigational groups was compared. A statistically reliable prolongation of the survival time of rats from the investigational group (HpD+Gy) was noted versus the survival time of the control rats without any treatment and the ones that underwent only HpD injection (Fig. 1). There were no statistically reliable differences between the survival time of rats from the Gy control group and the investigational group that had underwent GDT without any modification; however, the survival time of the latter group was longer.

As regards the HpD+Gy+glass investigational group, its survival time was similar to that of rats from the Gy control group.

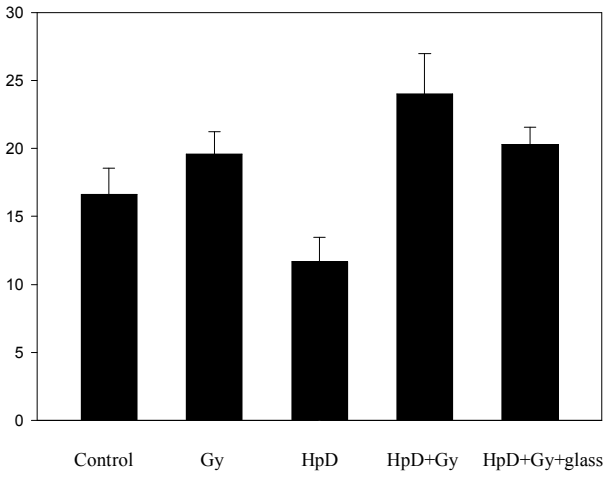


**Fig. 1.** Survival of Wistar rats with intracerebral C6 glioma after gammadynamic treatment. Values are mean (± SD, n = 6–9)

When the tumors were inoculated subcutaneously, a decrease of the tumor volume was noted in the first (HpD+Gy) investigational group immediately after the beginning of GDT when the tumors undervent a single dose of 2 Gy from radioactive $^{60}$Co, in contrast to the control groups in which the increasing volume of tumors was noted on day 5 of the experiment in rats without any treatment and in the ones that underwent only HpD, and on day 3 in the rats from the Gy control group (Fig. 2).

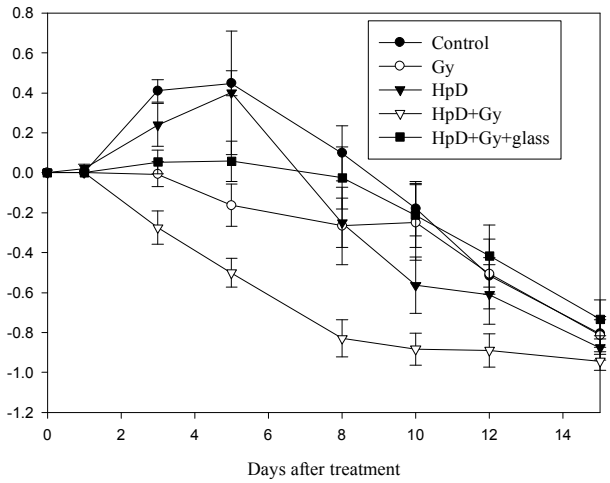


**Fig. 2.** Relative subcutaneous C6 rat glioma tumor growth after gammadynamic treatment. Values are mean (± SD, n = 6–9)

Later on the regression of the tumors was estimated in all the groups, but it was statistically reliably more significant than in all the control groups (there were statistically reliable differences in the relative tumor growth in the corresponding groups).

It is essential to point out that the first full regression of the tumor was fixed on the 10th day of the experiment in the first (HpD+Gy) investigational group, while in all other groups the first full regression of the tumor was fixed only on the 15th day of the experiment. As regards the HpD+Gy+glass group, a decrease of the volume of tumors was found in the Gy control group.

Summarizing the results of our experiment, we can state that C6 rat glioma is a suitable model for GDT investigation *in vivo*. Injections of $1\times10^7$ cells formed tumors in all cases without exception; the tumors were visible externally on day 5 and reached the volume of about 100–150 mm$^3$ 7–10 days after the cell implantation. Moreover, it was easy to measure the volume of the tumor without sacrificing the rat. We can thus save a lot of rat lives by employing this experimental system. Another advantage of this model is the rapid growth of C6 glioma at the subcutaneous site. An approximately 4-fold increase of the tumor volume was observed in the subcutaneous region between days 5 and 15 when the tumor volume reached its maximum. It was easy to monitor the tumor volume in the subcutaneous region.

As for soda-lime glass, we may regard it as a filter which can eliminate the essential wavelength (or wave-

lengths) of the γ rays spectrum capable of activating HpD. The detection of these wavelengths might allow us to find other ionizing radiation sources capable of activating some porphyrins more effectively.

At present, investigations of the spectrum of radioactive cobalt $^{60}$Co are under way. We are looking for quantitative and qualitative differences of the spectra, when the soda-lime glass is present or absent. We hope that in the future, in the process of the research, a new radiation source (or sources) will be suggested and the appropriate single and total ionizing radiation dosages adequate to those sources will be estimated.

This would offer the opportunities to develop such gammadynamic treatment methods when the one-time absorbent ionizing radiation dosage will be only ~0.2 Gy and the total ~0.6 Gy. This would allow researchers to apply effectively the gammadynamic treatment even to those patients, who are diagnosed with a very wide tumor outspread as well as to patients who had undergone maximal gamma ray dosage and the possibilities of their radical treatment are fully exhausted. Moreover, gammadynamic treatment could be applied repeatedly, independently of tumor localization.

If the porphyrins that accumulate in malignant tissues can be activated by different spectra of electromagnetic waves, new possibilities of ethiopathogenetic treatment of malignant tumors would open. This would allow to develop effectively sensitized tumor therapy itself, both while searching for new porphyrin sensitizers and looking for different lengths of the waves that activate porphyrins.

## CONCLUSIONS

C6 rat glioma is a suitable model for GDT investigation *in vivo*. Referring to our results, we may regard soda-lime glass as a filter which can eliminate the essential wavelength (or wavelengths) of the γ rays spectrum capable of HpD activation. The detection of these wavelengths would enable researchers to find other ionizing radiation sources capable of activating some porphyrins more effectively.

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**Laima Bloznelytė-Plėšnienė, Laimutė Rutkovskienė, Albinas Plėšnys**

## PORFIRINŲ SUŽADINIMAS SKIRTINGŲ SPEKTRŲ ELEKTROMAGNETINĖMIS BANGOMIS – NAUJOS PIKTYBINIŲ NAVIKŲ GYDYMO GALIMYBĖS

Santrauka

Iškėlus hipotezę, kad hematoporfirino darinius (HpD) galima sužadinti ne tik matoma šviesa bei ultravioletiniais spinduliais, bet ir trumpesnio bangos ilgio elektromagnetiniais virpesiais, atlikti eksperimentai, kurių metu skirtingų koncentracijų HpD tirpalai buvo veikiami tinkamo bangos ilgio matoma šviesa bei gama spinduliais.

Tam tikros koncentracijos HpD tirpalus apšvietus 405 nm mėlyna šviesa, plika akimi buvo matoma avietinė fluorescencija. Apšvitinus šiuos tirpalus gama spinduliais (vienkartine 2 Gy doze, radioaktyvių spindulių šaltinis – $^{60}$Co), avietinė fluorescencija išnykdavo. Gama spindulių veikimo metu, uždengus minėtus HpD tirpalus kalcio natrio silikatiniu stiklu, fluorescencija išlikdavo.

Atlikti eksperimentai, kurių metu žiurkėms, įskiepytoms glioma C6, buvo taikomas gamadinaminis gydymas. Suleidus HpD ir navikus paveikus mažomis gama spindulių dozėmis, stebėta statistiškai patikima jų regresija. Švitinant kalcio natrio silikatiniu stiklu uždengtus navikus, navikų augimas nebuvo stabdomas – gamadinaminio gydymo efektas išnykdavo. Šie rezultatai leidžia daryti išvadą, kad HpD aktyvina tie jonizuojančios radiacijos spektro spinduliai, kurie nepraeina pro kalcio natrio silikatinį stiklą.