New Efficient Nanostructural Near-IR Photosensitizer for Photodynamic Therapy of Malignancies based on Micellar Dispersion of Zinc octa-4,5-decylthio-octa-3,6-chlorophthalocyanine

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ABSTRACT

The work is devoted to the investigation of new nanostructural photosensitizer based on phthalocyanine derivative – zinc octa-4,5-decylthio-octa-3,6-chloro-phthalocyanine with absorption maximum at 730 nm.

Active substance was solubilized in micellar form based on non-ionic Pluronic-like surfactant. Nanostructural properties of dispersions were studied by means of correlation laser spectrometry. Optimization of content and proportion of components allowed us to achieve high stability of nanostructural properties of dispersion stored at room temperature. Dynamics and selectivity of photosensitizer accumulation in tumor and normal tissue were estimated *in vivo* from absorption spectra of sensitized tissue measured by diffuse reflectance approach.

Photosensitizer selectively accumulates in tumors. For PDT, they were irradiated using 732 nm laser with power density in a range of 100-300 mW/cm^2 for 20 min. Tumor growth inhibition for Ehrlich tumor exceeded 80%.

Keywords: photodynamic therapy, photosensitizer, phthalocyanine, micellar dispersion, absorption

1 INTRODUCTION

Methods of photodynamic therapy (PDT) and fluorescence diagnostics of malignancies are widely investigated for experimental and clinical oncology. High photodynamic efficiency, suitable spectral range, high quantum yield of fluorescence and photostability play the important role for the choice of photosensitizer for photodynamic therapy. Photosensitizers absorbing in the spectral range of 720-850 nm provide the greatest depth of photodynamic effect due to the minimal intrinsic absorbance of non-sensitized tissue in this range. Additional factor determining a choice of suitable spectral range for PDT is an availability of accessible sources of laser radiation in this range (in particular, laser diodes).

The work is devoted to the investigation of new perspective compound of alkylthio-substituted phthalocyanines series for development of photosensitizers of near-infrared range based on nanostructural dispersions.

2 MATERIALS AND METHODS

In this work we have conducted the *in vivo* investigation of zinc octa-4,5-decylthio-octa-3,6-chlorophthalocyanine [$(DecS)_8Cl_8PcZn$] which Q_y absorption maximum is located around 730 nm. The structure of the photosensitizer is shown on Fig. 1.

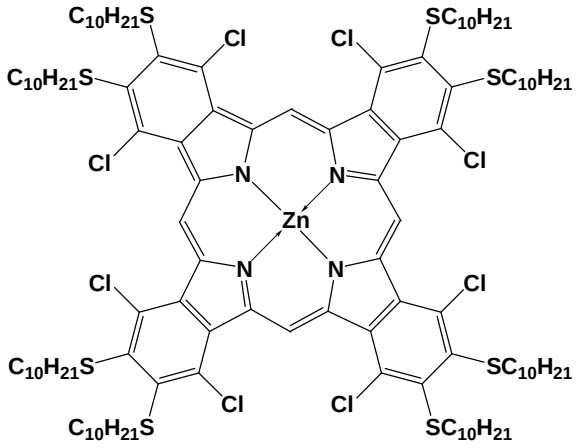

Figure 1: Chemical structure of zinc octa-4,5-decylthio-octa-3,6-chlorophthalocyanine.

For intravenous administration of hydrophobic $(DecS)_8Cl_8PcZn$, we have prepared its micellar dispersion on a base of non-ionic Pluronic-like surfactant. Particle size distribution was estimated by means of laser correlation spectrometry method using LCA-3 (Russia) and NICOMP CW-380 (USA) devices. These studies have shown that micelles had uniform size distribution with mean size in a range of 210-220 nm remaining stable for long time of storage under room temperature (Fig. 2).

The examination of sensitized biological tissue and photodynamic treatment were performed on F_1 hybrid mice bearing Ehrlich (ELD) tumor inoculated intramuscularly on right flank 4-5 days before administration of photosensitizer.

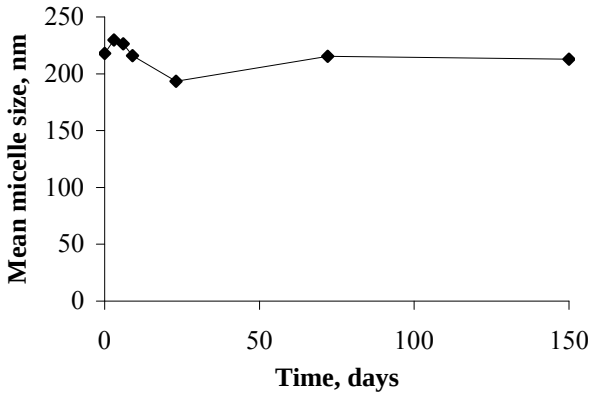

Figure 2: Dynamics of micelle size distribution of micellar dispersion of $(DecS)_8Cl_8PcZn$. Size distribution is homogeneous for all time points.

Dynamics and selectivity of $(DecS)_8Cl_8PcZn$ accumulation in tumor and normal tissue were estimated *in vivo* by absorption spectra using diffuse reflectance spectroscopy approach with spectroanalyzer LESA-01-Biospec (Russia) [1], upon intravenous administration of photosensitizer at doses of 0.5-4.0 mg/kg.

For the photodynamic therapy with $(DecS)_8Cl_8PcZn$ we have irradiated tumors area using diode laser LPhT-730-Biospec (Russia) emitting at 732 nm with power density up to 300 mW/cm^2. $(DecS)_8Cl_8PcZn$ was administered at dose of 2 mg/kg. Irradiation was started 4-5 hours after administration of photosensitizer and had duration of 20 minutes. Efficiency of photodynamic treatment was estimated by monitoring the inhibition of growth of tumor volume in treated animal groups in comparison to control group. Tumor growth inhibition index (TGI, %) was calculated from the following expression:

$$TGI\ (\%) = \frac{(V_C - V_T)}{V_C} \times 100\ ,$$

where V_C – average tumor volume in control group (cm^3), calculated as half-product of three orthogonal measurements of tumor; V_T – average tumor volume in PDT treated group (cm^3).

Groups for treatment consisted of 6 mice each, control groups consisted of 6 to 8 animals.

3 RESULTS AND DISCUSSION

$(DecS)_8Cl_8PcZn$ possesses considerable absorbance *in vivo* in the spectral range of minimum intrinsic absorption of biological tissue with its spectral maximum located around 725-730 nm (Fig.3), however it does not possess fluorescent ability. Therefore, the dynamics and selectivity of accumulation of $(DecS)_8Cl_8PcZn$ were estimated from integral intensity of its absorption spectra *in vivo*.

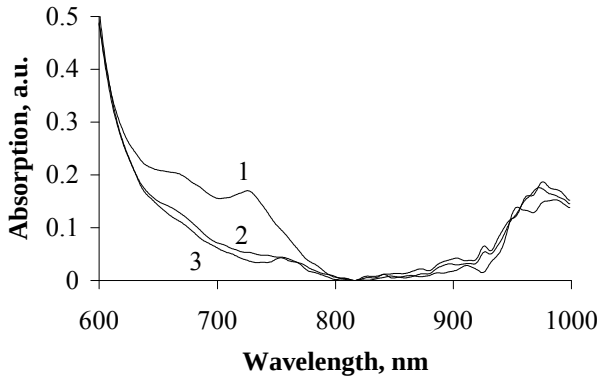

Figure 3: Absorption spectra of sensitized tumor (1) and normal tissue (2) 4 hours after intravenous administration of $(DecS)_8Cl_8PcZn$ in micellar dispersion at dose of 2 mg/kg. (3) – intrinsic absorption spectrum of non-sensitized tissue.

Our studies have shown that sufficiently high content of investigated photosensitizer in tumor was observed at 4-5 hours after its administration. $(DecS)_8Cl_8PcZn$ selectively accumulated in tumor and completely cleared from normal tissue by 6-8 days after administration (Fig. 4).

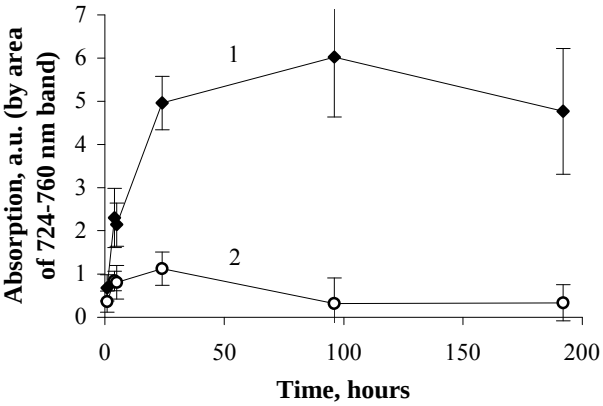

Figure 4. Accumulation of photosensitizer in Ehrlich tumor (1) in comparison to normal tissue (2) after intravenous administraton of $(DecS)_8Cl_8PcZn$ in micellar dispersion at dose of 2.1 mg/kg.

For PDT studies with $(DecS)_8Cl_8PcZn$, tumor area was irradiated using diode laser LPhT-730-Biospec (Russia) emitting at 732 nm with power density 150 mW/cm^2 for 20 minutes (achieving light dose density of 180 J/cm^2), starting 4-5 hours after intravenous administration of photosensitizer at dose of 2 mg/kg. It was shown that PDT with $(DecS)_8Cl_8PcZn$ significantly (more than 83%) inhibits the growth of the Ehrlich tumor (Fig.5).

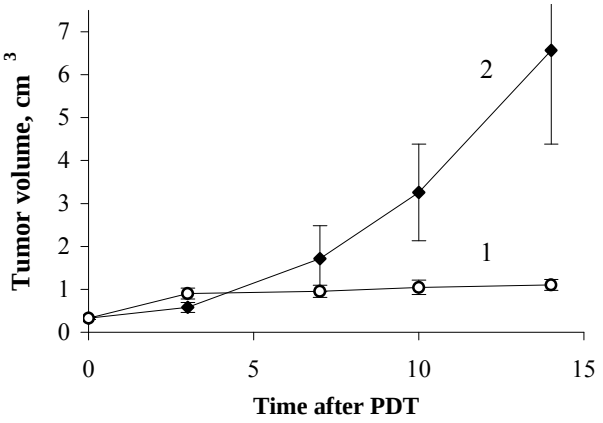

Figure 5. Growth inhibition of Ehrlich tumor after PDT with $(DecS)_8Cl_8PcZn$ administered in micellar dispersion at dose of 2 mg/kg (1) in comparison to non-treated group (2). Tumor was treated 4-5 hours after photosensitizer administration using 732 nm laser with power density of 150 mW/cm^2 for 20 min.

4 CONCLUSION

The preliminary results obtained in our investigation have shown that micellar dispersion of $(DecS)_8Cl_8PcZn$ shows high stability of its nanostructural parameters at room temperature. *In vivo* study of this nanostructural photosensitizer has demonstrated that it selectively accumulates in tumor, rather quickly clears from normal tissue and exhibits high photodynamic efficiency on relatively large model tumors.

5 ACKNOWLEDGEMENTS

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