Preliminary studies of new photosensitizer for photodynamic treatment of melanoma based on nanostructural forms of Bacteriochlorin p N-alkoxycycloimide alkoxoxyxyme methyl ester


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ABSTRACT

Method of photodynamic therapy (PDT) of malignancies is actively used in experimental and clinical oncology. This work is devoted to investigation of Bacteriochlorin p N-alkoxycycloimide alkoxoxyxyme methyl ester which possesses its absorption band at 795-800 nm. We have estimated the applicability of PDT with this photosensitizer to treat melanoma.

We have developed nanostructural forms of the substance – micellar dispersions on a base of 4% aqueous solution of Cremophor EL and Emuxol-268 and PEG-liposomal dispersion.

In all forms photosensitizer was selectively accumulated in tumor in comparison to normal tissue. PDT on BDF1 mice bearing B16 melanoma have shown high therapeutic efficiency with inhibition of tumor growth up to 91%, increase of animal lifespan exceeded 60%. Complete recovery was achieved for 35% of animals.

Keywords: photodynamic therapy, bacteriochlorin, nanostructural forms, melanoma

1 MATERIALS AND METHODS

In this work we have performed in vivo investigation of Bacteriochlorin p N-alkoxycycloimide alkoxoxyxyme methyl ester. Substance was synthesized by modification of bacteriochlorophyll a, obtained from purpuric bacteria Rhodobacter capsulatus [1,2]. We have developed and studied several nanostructural injectable forms of the substance – micellar dispersions on a base of 4% aqueous solution of Cremophor EL and Emuxol-268 (pluronic-like surfactant) and PEG-liposomal dispersion.

Nanostructural parameters of all dispersions were estimated using NICOMP-380 system (USA). Main vesicle size for Cremophor and Emuxol-based micellar dispersions and PEG-liposomal dispersion was in a range of 12-15 nm, 180-260 nm and 100-180 nm, respectively.

Investigations of accumulation of photosensitizer in tumors and normal tissue of mice upon administration in different injectable forms were performed by absorbance spectroscopy of sensitized tissue in vivo using diffuse reflectance approach with spectrophotometer "LESA-01-Biospec" (Biospec, Russia) [3]. Dynamics and selectivity of accumulation of photosensitizer in tumor and normal tissue was studied on F1 (C57BL/6 × CBA hybrid) mice bearing Ehrlich (ELD) tumor, inoculated intramuscularly on right hind leg. Photosensitizer dispersions were administered to animals intravenously 4 days after inoculation of tumor according the dose of substance 4 mg/kg.

Photodynamic activity of Bacteriochlorin p N-alkoxycycloimide alkoxoxyxyme methyl ester was investigated on BDF1 (C57BL/6 × DBA/2 hybrid) mice bearing B16/F10 melanoma inoculated intramuscularly on right hind leg 7 days prior to treatment. Photosensitizer was administered in different forms according to the substance dose up to 1 mg/kg.

To perform the photodynamic therapy we have illuminated tumors using LPHT-800-01-Biospec laser emitting at 797 nm (Biospec, Russia), applying power density up to 250 mW/cm2, starting the treatment 1-2 hours after administration of photosensitizer dispersions, when the level of its accumulation in tumor upon administration in each respective form was close to maximum.

Photodynamic efficiency of photosensitizers 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:

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

where $V_C$ – average tumor volume in control group (cm³), calculated as half-product of three orthogonal measurements of tumor; $V_T$ – average tumor volume in PDT treated group (cm³). Groups for treatment consisted of 6 mice each, control group consisted of 6 to 8 animals.

2 RESULTS AND DISCUSSION

Results of investigation of accumulation of Bacteriochlorin p N-alkoxycycloimide alkoxoxyxyme
methyl ester in tumor in comparison to normal tissue are shown on Fig.1 A-C.

In all forms of administration photosensitizer was efficiently and selectively accumulated in tumor achieving the maximum at 1-3 hours after injection, while it was cleared from the normal tissue down to detection threshold by 5-8 hours after administration. The selectivity index exceeded the value of 4.5:1 at the time of maximum accumulation for all developed forms and achieved the value 8-12:1 by 4-5 hours after administration.

Figure 1: Dynamics of optical absorption of sensitized tumor (1) and normal tissue (2) after i.v. administration of Bacteriochlorin \( \mu \) N-alkoxyxcycloimide alkoxyoxyme methyl ester at dose of 4 mg/kg in form of (A) liposomal dispersion, (B) Cremophor EL based micellar dispersion, (C) Emuxol-268 based micellar dispersion.

PDT with nanostructural forms of Bacteriochlorin \( \mu \) N-alkoxyxcycloimide alkoxyoxyme methyl ester was performed on BDF1 hybrid mice bearing B16 melanoma. Photosensitizer preparations were intravenously administered 7 days after intramuscular inoculation of tumor at doses up to 1 mg/kg. Sensitized tumors were irradiated by LPhT-800-Biospec laser (Russia) with maximum at 797 nm 1-2.5 hours after injection of photosensitizer with power density up to 200 mW/cm² for 20 minutes.

Example of results for Bacteriochlorin \( \mu \) N-alkoxyxcycloimide alkoxyoxyme methyl ester administrated in form of micellar dispersion based on Cremophor EL at dose of 1 mg/kg is shown on Fig.2 A,B. Photosentitizer have shown high efficiency for PDT treatment of melanoma with inhibition of tumor growth achieving 91%, while increase of animal lifespan exceeded 60% and for 35% of animals was achieved complete recovery.

Figure 2: Dynamics of growth of B16 melanoma (A) and tumor growth inhibition (B) after PDT treatment using Cremophor-based micellar dispersion of Bacteriochlorin \( \mu \) N-alkoxyxcycloimide alkoxyoxyme methyl ester at dose of 1 mg/kg (1) in comparison to non-treated control group (2).
CONCLUSION

The results obtained in preliminary investigation have shown that Bacteriochlorin p N-alk oxy cyclo imide alkoxy oxyme methyl ester in developed nanostructural forms demonstrates high photodynamic efficiency and fast clearance from normal tissue. Photosensitizer seems to be promising for PDT of pigmented tumors, such as melanoma.

REFERENCES


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