Influence of Liposome Size on Accumulation in Tumor and Therapeutic Efficiency of Liposomal Near-IR Photosensitizer for PDT based on Aluminum hydroxide tetra-3-phenylthiophthalocyanine

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ABSTRACT

Size of nanoparticles is one of main factors determining the efficiency of nanostructural antitumor drugs. This work was performed to estimate the influence of liposome size on level and selectivity of photosensitizer accumulation in tumor and efficiency of photodynamic treatment (PDT) using photosensitizer Tiosens (liposomal form of $(PhS)_4PcAlOH$) with absorption maximum at 720 nm.

It was shown that administration of Tiosens dispersions with liposome size range of 75-130 nm results in level and selectivity of photosensitizer accumulation in tumor noticeably higher in comparison to the ones with 180-450 nm size. Liposomes of size less than 50-60 nm show high accumulation both in tumor and normal tissue resulting in less selectivity of accumulation and prolonged retain in normal tissue. PDT using Tiosens dispersion of size below 150 nm causes efficient tumor growth inhibition (TGI) achieving 80%, while use of dispersion of larger liposome size gives only moderate TGI of about 53%.

Keywords: photodynamic therapy, photosensitizer, phthalocyanine, liposome size distribution, fluorescence.

1 INTRODUCTION

The level and selectivity of photosensitizer's accumulation in tumor as well as the rate of its clearance from the normal tissue are important characteristics for photodynamic therapy (PDT) and fluorescent diagnostics. They influence both the efficiency of PDT treatment and probability of damage of normal tissue adjacent to the pathological zone as well as increased skin photosensitivity after the treatment. On the other hand, the same characteristics are also significant for optimization of photosensitizer's dosage and regime of laser irradiation.

In the last years there is serious interest for nanostructural systems of drug delivery which included active work for development of liposomal and micellar forms of photosensitizers.

Dynamics of accumulation of nanostructural forms of photosensitizers depends on size of nanostructures. One of factors of this dependence is trapping of nanoparticles by reticuloendothelial system. In many cases this problem is mostly solved by including into composition of nanostructure polyethylenglycole molecules (so called PEGylating). For example, such method is widely used for liposomes.

The size of nanostructural photosensitizers also affects the interaction of nanostructures with defects of endothelial layer of tumor neovasculature [1-4]. Previous investigation of bio-distribution of PEGylated liposomes of the same composition (DSPC:CH:DSPE-PEG = 10:10:1 molar) but various sizes containing colloidal gold on different tumor models, using electronic microscopy method, have shown that size dependence of their accumulation in tumor is not monotone, with its optimum in a range of 80-120 nm (see Fig.1, [1]).

Investigations of different tumors using electronic microscopy allowed to show the defects of endothelial layer of tumor neovasculature (black arrows on microphoto, Fig.2) of human colon carcinoma LS 174T with their size in a range of 200-2000 nm.

This allows to assume increased permeability and extravazation of nanoparticle drugs in area of neovasculature endothelium defects (shown by white arrows on microphoto, Fig.3) and subsequent enhanced accumulation in tumor, in dependence of nanostructure size (mean liposome size in this example – 126±35 nm).

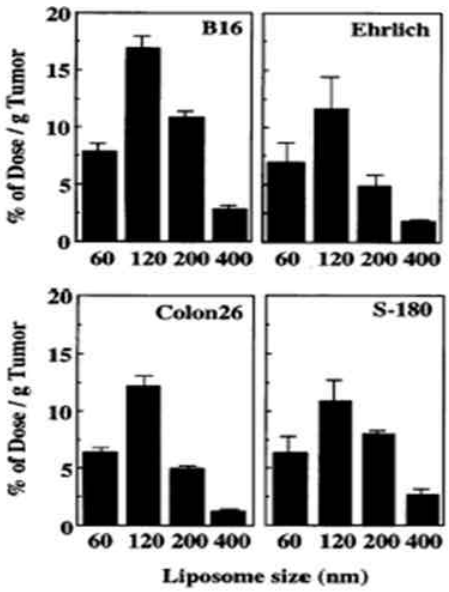

Figure 1. Accumulation of gold-containing PEG-liposomes in dependence on liposomal size in various solid tumors 6 hours after intravenous administration [1].

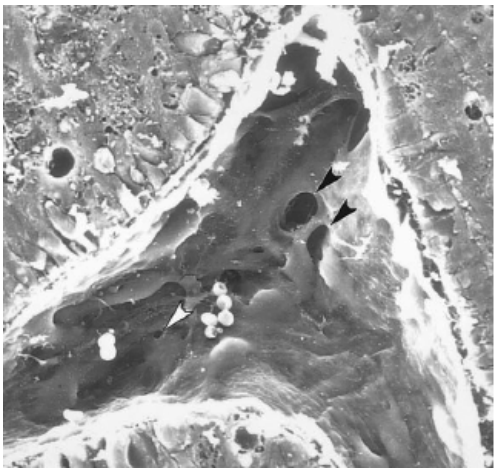

Figure 2. Defects in the endothelial monolayer of tumor vessels of human colon carcinoma LS 174T [2].

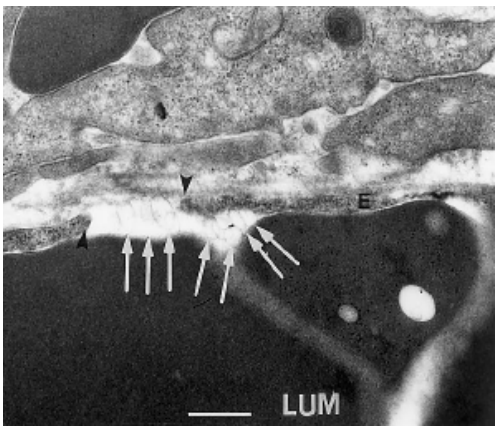

Figure 3. Extravasation of liposomes via the endothelial defect [1].

A number of investigators have studied the influence of liposome size on level of accumulation of drugs in tumors [1, 5, 6] and have shown that the most efficient accumulation is provided by nanocarriers with mean size from 90 nm [5] to 160-220 nm [6]. Additionally, accumulation level depends on a number of other factors including the tumor type and its characteristic vessel endothelium defects (see examples in Table 1):

Tumor species	Endothelial defects size range, nm
Mice breast carcinoma MCa-IV	1200-2000
Shionogi breast tumor	200-380
Colon carcinoma LS 174T	400-600

Table 1: Characteristic sizes of the endothelial defects of tumor neovasculature for different tumor models [2].

Because of this it is important for technological optimization and development of new photosensitizers to perform the experimental estimation of the nanocarrier size influence on the level and selectivity of accumulation of nanostructured photosensitizers on chosen tumor models.

In this work we have studied the influence of liposome size distribution on level and selectivity of accumulation in tumor and normal tissue and efficiency of photodynamic treatment using near-IR photosensitizer Tiosens (liposomal form of aluminum hydroxide tetra-3-phenylthiophthalocyanine [$(PhS)_4PcAlOH$]) [7] administered in form of liposomal dispersions with different size distribution in a range of less than 50 nm to 450 nm.

2 MATERIALS AND METHODS

Liposomal dispersion of Tiosens was prepared on a base of lecithin EPCS (Lipoid, Germany), cholesterol (Sigma, Japan) and mPEG2000-PE (Lipoid, Germany), using Bangham approach, with hydrophobic substance of Tiosens [$(PhS)_4PcAlOH$] included into lipid bilayer. Molar ratio of components in respective order was 205:48:12:1.

Liposome sizes were decreased and unified by extrusion of liposomal dispersion using high pressure homogenizer EmulsiFlex-C5 (Avestin, Canada) and hand-operated Mini-Extruder (Avanti Lipids, USA) equipped with Nucleopore (Whatman, USA) membranes of different pore size. Size distribution of liposomal dispersions after the extrusion was determined by laser correlation spectroscopy using Nicomp-CW380 (USA) and LCA-3 (Russia) devices.

In vivo investigations were performed using F_1 ($C_{57}B1/6$ x DBA/2 hybrid) mice bearing Ehrlich tumor (ELD) intramuscularly inoculated to right flank 4-5 days before experiment. Photosensitizer was administered via the tail vein at dose of 6 mg/kg. Level and selectivity of accumulation of Tiosens in tumor were studied by *in vivo* fluorescent spectroscopy method [8] using fiber-optic spectroanalyzer LESA-01-Biospec (Biospec, Russia).

Photodynamic treatment of tumors sensitized with Tiosens was performed using diode laser LPhT-730-Biospec (Russia) emitting at 732 nm with power density of 400 mW/cm^2. Photosensitizer was administered at dose of 6 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

Size dependence of level and selectivity of accumulation of liposomal photosensitizer in tumor in comparison to normal tissue may be connected to several main factors. First of all, circulation time of liposomes in the blood pool decreases due to their capture by reticulo-endothelial system (RES) which withdraws liposomes despite their PEGylation, to the larger extent the bigger are liposomes. Second, efficiency of extravasation of liposomes via the endothelial layer of tumor neovasculature largely depends on relation between size of liposomes and characteristic size of gaps in tumor naovasculature endothelium.

Accumulation in tumor of photosensitizer administered in liposomes of large size range (180-450 nm) is inefficient both due to decreased time of circulation in the blood pool and because of inefficient extravasation. Selectivity of accumulation is rather low, index of selectivity in the short time range up to 5-6 hours after administration reaches the values up to 2-3. Slightly larger level as well as relatively high selectivity of accumulation in tumor is achieved only in time range of 1-4 days after administration. On the other hand, photosensitizer administered in large liposomes quickly clears form the normal tissue; its level in skin decays to detection threshold by 7 days after administration (Fig.4, lines 1, 4).

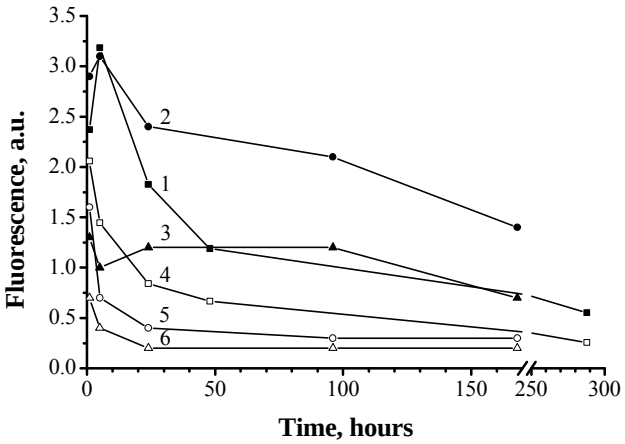

Figure 4. Accumulation of Tiosens in tumor (1,2,3) and normal tissue (4,5,6) after administration in form of dispersion with liposome size range of 20-60 nm (1,4); 75-130 nm (2,5); 180-450 nm (3,6).

In the range of sizes we consider optimal (75-130 nm) accumulation of photosensitizer in tumor is high due to both relatively long time of circulation in blood pool and efficient extravasation via the tumor neovasculature endothelium. Selectivity of accumulation is also high, index of selectivity by 3 hours after administration exceeds the value of 3. At the same time, photosensitizer clears from the normal tissue relatively fast and is content in the skin by 7-8 days after administration is only slightly higher than for large liposomes (Fig.4, lines 2, 5).

Further decrease of size of liposomes (less than 50-60 nm), however, does not give much advantage in liposomes extravasation and photosensitizer's accumulation in tumor which stays at about the same level achieved at 4-6 hours after administration of Tiosens. At the same time, probability of liposome capture by RES is noticeably decrease, thus increasing time of circulation of liposomes in the blood pool. Because of this, the selectivity of accumulation of very small liposomes in tumor is less than for liposomes of 75-130 nm size and limited by the value of about 2 at the time of maximum photosensitizer accumulation in tumor, and prolonged retaining of high content of photosensitizer in the normal tissue is observed (Fig.4, lines 3, 6). It leads to prolonged phototoxicity and larger risk of photodamage of skin, causing the patient to longer maintain special regime of limited light and decreasing his life quality.

Efficiency of photodynamic treatment using Tiosens administered in large liposomes is moderate; tumor growth inhibition (TGI) achieves values of 53% (Fig.5, line 1). In order to maximize the therapeutic efficiency and minimize the traumatization of adjacent normal tissue under these conditions it can be recommended to perform irradiation of tumors at least 24 hours after administration of photosensitizer.

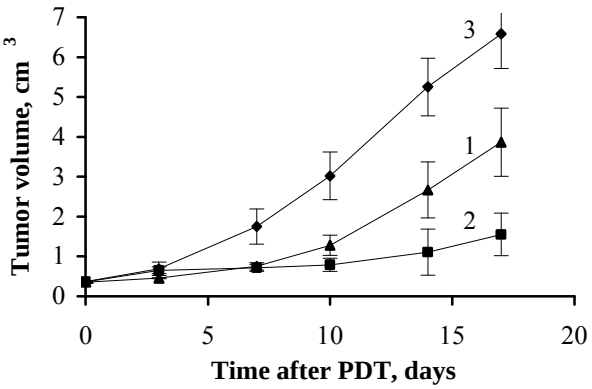

Figure 5. Growth inhibition of Ehrlich tumor after PDT with Tiosens administered in at dose of 6 mg/kg in dispersions of 180-450 nm (1) and 75-130 nm (2) liposome size range in comparison to control non-treated group (3). Tumors were treated 4-5 hours after photosensitizer administration using 732 nm laser with power density of 400 mW/cm^2 for 20 min.

PDT using liposomes of optimum size can be recommended to perform in a time range of 4 to 24 hours after aministration of photosensitizer. It provides high therapeutic efficiency with TGI up to 80% (Fig.5, line 2) and minimal traumatization of adjacent tissue.

Finally, while photodynamic treatment using Tiosens liposomes of small size range (20-60 nm) efficiently destructs the tumor, at the same time, due to high level of photosensitizer in normal tissue, the latter also undergoes noticeable photodynamic influence which causes heavy damage for the irradiated organ (hind leg under conditions of this work).

4 CONCLUSION

Results of *in vivo* investigation have shown that administration of Tiosens dispersions with liposome size range of 75-130 nm results in level and selectivity of photosensitizer accumulation in tumor noticeably higher in comparison to the ones with 180-450 nm size. Liposomes of size less than 50-60 nm show high accumulation both in tumor and normal tissue resulting in less selectivity of accumulation and prolonged retain in normal tissue. PDT using Tiosens dispersion of size below 150 nm causes efficient tumor growth inhibition achieving 80%, while use of dispersion of large liposome size gives moderate or low TGI of about 53%.

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