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Effect of delivery system on the pharmacokinetic and phototherapeutic properties of bis(methyloxyethyleneoxy)silicon-phthalocyanine in tumor-bearing mice

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Abstract

A Si(IV)-phthalocyanine bearing two methoxyethyleneglycol axial ligands bound to the central metal ion (SiPc) has been prepared by chemical synthesis and analyzed for its phototherapeutic activity after administration in a Cremophor or liposome formulation to C57Bl/6 mice bearing a subcutaneously transplanted Lewis lung carcinoma (LLC). The maximum drug accumulation in the tumor is found at 24 h after intraperitoneal injection, independent of the delivery system. However, the tumor concentration of SiPc in the Cremophor formulation is about two-fold higher, while the drug concentration in liver and skin shows similar trends with the two delivery systems. The drug accumulation and retention in the brain is much larger when using Cremophor emulsion. Photodynamic therapy (672 nm, 370 mW m^{-2}, 360 J cm^{-2}) at 24 h after the injection of Cremophor emulsion- or DPPC liposome-formulated SiPc causes a very efficient and similar response for the LLC (~8 versus 22 mm mean tumor diameter for the control groups at 21 days after phototreatment). These very promising effects, obtained both at higher and lower tumor drug concentrations, clearly demonstrate the potential phototherapeutical activity of the newly synthesized SiPc.

Keywords: Axial ligands; Cremophor EL; Liposomes; Pharmacokinetics; Photodynamic therapy; Si(IV)-phthalocyanine; Tumor model

1. Introduction

Although photodynamic therapy (PDT) is becoming an established modality for the treatment of a variety of solid tumors, it is generally recognized that its widespread clinical application would be facilitated by the definition of improved phototherapeutical protocols. For this purpose, several approaches are being explored for developing the so-called 'second-generation' tumor photosensitizers as well as tumor-specific carriers for the systemically administered photosensitizers [1–3]. In particular, great attention is being focused on phthalocyanines, which show absorption bands above 660 nm with molar extinction coefficients at least two orders of magnitude larger than that typical of HpD in the clinically useful 600–700 nm region. Moreover, many phthalocyanines are characterized by a high level of hydrophobicity. This property is considered to increase the affinity of photosensitizers for neoplastic tissues [3]. The poor water solubility of most phthalocyanines, however, often prevents their direct injection into the bloodstream. To overcome this problem, polar substituents such as sulphonic acid, carboxylic acid and hydroxyl groups were added to the peripheral positions of the macrocycle. Interesting possibilities were opened when the hydrophilicity of the macrocyclic photosensitizers was increased through the coordination of suitable axial ligands, such as Si(IV), to the central metal ion [4]. It was also demonstrated that such ligands can improve the physicochemical properties [5] and the pharmacokinetic behavior of PDT photosensitizers [6]. Alternatively, hydrophobic photosensitizers can be administered after incorporation into different delivery systems such as liposomes, oil emulsions

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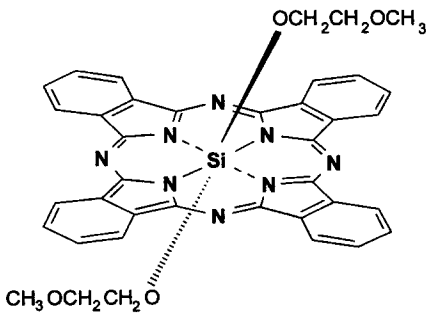

Fig. 1. Chemical structure of Si(IV)-methoxyethyleneglycol-phthalocyanine (SiPc).

or covalent complexes with monoclonal antibodies directed against antigens located on the surface of malignant cells.

In the present work we describe the pharmacokinetic and phototherapeutic properties of a silicon(IV)-phthalocyanine bearing two axial ligands to the centrally coordinated metal ion. The chemical structure is shown in Fig. 1. The study was carried out in a comparative manner using two different delivery systems.

2. Materials and methods

2.1. Chemicals and instruments

Cremophor EL and DL-α-dipalmitoyl-phosphatidylcholine (DPPC) were purchased from Sigma (Germany) and dichlorosilicon-phthalocyanine from Aldrich. All other chemicals were analytical grade reagents. UV–Visible absorption spectra of the synthesized Si(IV)-methoxyethyleneglycol-phthalocyanine (SiPc) were recorded on a Shimadzu UV-3000 double-beam spectrophotometer. The fluorescence emission spectra were taken by means of a Perkin–Elmer LS-5 spectrophotofluorimeter equipped with a red-light-sensitive phototube.

For the synthesis of bis(methyloxyethyleneoxy)silicon-phthalocyanine, dichlorosilicon-phthalocyanine (61 mg, 0.1 mmol) and ethyleneglycolmonomethylether (80 μl, 1 mmol) in 10 ml dry distilled *N,N*-dimethylformamide (DMF) were heated under reflux for 2 h. DMF and the excess ethyleneglycolmonomethylether were distilled off in vacuo. The residue was treated with water in a Soxhlet apparatus and afterwards purified twice by flash chromatography on Si60 using chloroform as the eluent. Yield: 41 mg (60%). UV–Vis ($CHCl_3$) λ (nm): 672, 638, 605, 353. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm): 9.65 (m, 8H, ar), 8.35 (m, 8H, ar), 1.75 (s, 6H, CH_3), 0.25 (t, 4H, CH_2), −1.90 (t, 4H, CH_2). MS (DCI neg., NH_3, 8 mA/s) *m/z*: 692 (21%), 691 (63%), 690 (100%, M^+). IR (KBr) ν (cm^{-1}): 3065, 2962, 2924, 2855, 1613, 1520, 1471, 1428, 1336, 1290, 1261, 1122, 1081, 911, 803, 759, 734, 574, 531.

2.2. Cremophor EL formulation

For the Cremophor emulsion drug formulation a modified procedure of Soncin et al. [7] was followed. Typically, 1.5 mg of SiPc was added to 0.3 ml of Cremophor EL and sonicated until the drug was completely dissolved; after addition of 0.09 ml absolute ethanol under controlled temperature, the suspension was diluted to a volume of 7.5 ml by addition of physiological solution and filtered through a 0.45 μm filter. The SiPc concentration in the Cremophor emulsion was estimated upon dilution in DMF by measuring the absorbance at 672 nm ($\varepsilon = 5.10^5\ M^{-1}\ cm^{-1}$).

2.3. Liposome preparation

Liposomes were prepared as previously described (1.0 mg SiPc in tetrahydrofuran, 70 mg DPPC in chloroform, suspension of the lipid film in 10 ml aqueous phosphate buffer and sonication) [8,9]. SiPc was incorporated into liposomal vesicles in a monomeric form, as demonstrated by the shape of the absorption spectrum (not shown). The concentration of the sensitizer in the liposomal dispersion to be systemically injected was calculated in the same way as in the case of Cremophor emulsion system.

2.4. Animals and tumor model

Pathogen-free female C57Bl/6 mice, 6–8 weeks of age, were purchased from the Experimental Animal Production Area (National Oncology Centre, Sofia, Bulgaria). In this study Lewis lung carcinoma (LLC), which is syngenic to the C57Bl/6 mice, was used. LLC cells were injected subcutaneously into the right hind leg of the mice with 0.2 ml of sterile suspension containing at least 2×10^6 viable cells per ml. Seven days after the implantation, the tumor reached an outer diameter of between 0.3 and 0.5 cm. PDT was applied at this stage of growth when the degree of spontaneous tumor necrosis was negligible.

2.5. Pharmacokinetic studies

Seven days after transplantation, the tumor-bearing mice (three animals per group) were intraperitoneally (i.p.) injected with 0.5 mg kg^{-1} b.w. SiPc incorporated into a Cremophor EL emulsion or liposome vesicles. The i.p. injection route was selected to minimize the risk of anaphylactic reactions, which may occur in case of intravenous injection of Cremophor [10]. At fixed times after dosing, the mice were sacrificed and tumor, peritumoral skin, liver and brain were collected. Then the SiPc concentration in each specimen was determined through fluorescence spectroscopy measurements after chemical extraction [8]. The fluorescence excitation was carried out at 605 nm, while the emission spectra were recorded in the 640–700 nm region.

2.6. Photodynamic therapy studies

When the tumor diameter was in the 0.3–0.5 cm range, groups of five tumor-bearing mice were i.p. injected with SiPc (0.5 mg kg^{-1} b.w.) in a Cremophor emulsion or DPPC

liposomes. The tumors were illuminated at 24 h post injection with 672 nm light from an argon-pumped dye laser (Spectra Physics, USA). The central part of the beam from the optical fiber was selected by a circular aperture in order to produce an irradiation spot with a constant fluence rate. According to previous protocols [9,11], in all cases the fluence rate was selected to be 370 mW cm^{-2} for a fluence of 360 J cm^{-2}. The effectiveness of the treatment was evaluated by comparing the mean tumor diameter and the tumor growth rate of the treated (drug-injected and irradiated) groups of mice with those of the control group (without drug and irradiation).

3. Results

3.1. Pharmacokinetic studies

The biodistributions of SiPc in LLC after incorporation in Cremophor emulsion or DPPC liposomes showed significant differences (Table 1). In particular, the SiPc reached maximal tumor concentrations (up to 2.16 μg g^{-1} tumor tissue) at 24 h post injection in Cremophor formulation as compared with maximal values of about 0.83 μg g^{-1} for the liposome-incorporated drug.

On the other hand, the amount and time dependency of photosensitizer accumulation in liver and skin were comparable for both delivery systems (Table 1). As a consequence there was a higher selectivity of tumor targeting by the Cremophor-delivered phthalocyanine photosensitizer as expressed by the ratio between the drug concentration in the tumor and the skin (peritumoral tissue).

Important differences in the pharmacokinetic behavior of SiPc towards the brain tissue were also detected for the two delivery systems, with a longer retention for the Cremophor formulation (Table 1) as well as a higher accumulation for post-injection times above 24 h.

Table 1
Recoveries of SiPc from selected tissues of C57Bl/6 mice bearing LLC at different times after i.p. injection of 0.5 mg kg^{-1} of phthalocyanine in Cremophor or liposome delivery system. Recoveries are expressed as micrograms of SiPc per gram of tissue and represent the average (±standard deviation) of data obtained for three independently analysed mice at each time

Tissue	Time (h)	Cremophor	DPPC liposomes
Tumor	3	0.70±0.15	0.20±0.05
	15	2.06±0.08	0.64±0.06
	24	2.16±0.63	0.83±0.15
	48	1.45±0.30	0.71±0.43
	72	0.37±0.06	0.30±0.07
Liver	3	1.98±0.85	2.28±0.89
	15	1.83±1.07	1.93±0.12
	24	1.81±0.46	1.87±0.29
	48	1.13±0.05	1.61±0.41
	72	1.05±0.16	1.50±0.25
Brain	3	0.19±0.01	0.41±0.11
	15	0.25±0.11	0.43±0.18
	24	0.45±0.05	0.12±0.03
	48	0.88±0.02	n.d.
	72	0.33±0.06	n.d.
Skin	3	0.38±0.14	0.36±0.05
	15	0.50±0.09	0.38±0.11
	24	0.41±0.11	0.35±0.12
	48	0.39±0.10	0.42±0.08
	72	0.11±0.04	0.10±0.05

n.d. = not detected.

3.2. Photodynamic therapy

Fig. 2 shows the time dependency of the mean tumor diameter after sensitization with 0.5 mg kg^{-1} b.w. SiPc formulated in Cremophor EL or DPPC liposomes. The irradiations were performed at the λ_{max} of SiPc (672 nm) and at 24 h after i.p. administration, since this post-injection time corresponded to the largest phthalocyanine accumulation in the neoplastic tissue. Clearly, the tumor responses to PDT were quite similar in the case of the two delivery systems. The mean tumor diameter increased rather slowly up to the sixth to seventh day post PDT and was still about three times lower than that of the control animals at the end of our observation period.

The tumor regrowth delay caused by PDT for the two delivery vehicles is given in Table 2. Clearly, a decrease in the rate of tumor growth was obtained for both delivery systems.

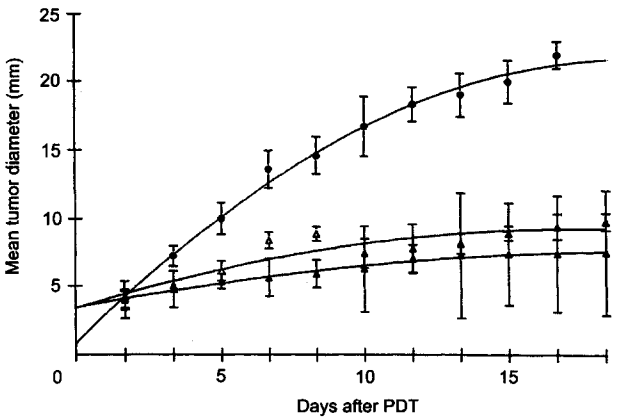

Fig. 2. Tumor growth curves (mean tumor diameter from five animals ± SD) for mice treated with Cremophor (▲) or DPPC liposome (△) formulated SiPc (0.5 mg kg^{-1} b.w.). Irradiation was performed at a fluence rate of 370 mW cm^{-2} for a total light dose of 360 J cm^{-2}. The control group (●) includes untreated and unirradiated mice.

Table 2
Effect of delivery system on tumor regrowth after PDT with SiPc (0.5 mg kg^{-1})

Formulation with	Growth time [a] (days)	Regrowth delay [b] (days)
Cremophor EL	10.50±4.8	6.9±4.32
DPPC liposome	9.75±3.56	5.2±3.08

[a] The time interval (±SD) for the tumor to grow to a mean diameter of 8 mm.
[b] Difference between the growth times for PDT-treated and control mice.

4. Discussion

The newly synthesized SiPc derivative appears to be a suitable phototherapeutic agent for use in PDT of tumors. This compound exhibits a high affinity for an experimental tumor model and, upon photoactivation with a deep-tissue-penetrating red-light wavelength, induces an efficient degree of tumor necrosis, leading to a significant delay in the rate of post-irradiation tumor regrowth. In this connection, SiPc represents a novel addition to the class of metal-phthalocyanines that are being investigated as second-generation tumor photosensitizers [12]. The high molar extinction coefficient and photostability of this drug, as well as its relatively large quantum yield for the generation of singlet oxygen [13], endow SiPc with further interesting properties.

In spite of the presence of two hydrophilic axial ligands, SiPc shows a poor water solubility, probably because of the strong hydrophobic contribution given by the extended tetraazaisoindole macrocycle. As a consequence, it is necessary to preincorporate the phthalocyanine into lipid-type delivery systems in order to proceed to its systemic administration.

Two different vehicles were tested in our experiments, namely, a Cremophor oil emulsion and DPPC liposomal vesicles. The pharmacokinetic studies clearly indicate that the former vehicle yields a significantly larger selectivity of tumor targeting, mainly as a consequence of an enhanced accumulation in the malignant lesion; no appreciable difference in the SiPc uptake by selected healthy tissues is observed with Cremophor or DPPC liposomes. The greater recovery of Cremophor-delivered SiPc can be correlated with the tendency of this vehicle to release the associated photosensitizing drug to serum low-density lipoproteins (LDLs) in a highly preferential amount, while DPPC liposomes transfer the drug to all the components of the lipoprotein class in aliquots that are proportional to the relative concentration of the individual proteins [14,15]. LDLs are known to display a preferential interaction with a variety of neoplastic cells through a receptor-mediated endocytotic process [16]: these cells often express an increased number of LDL receptors as compared with normal cells.

In spite of the markedly lower amount of SiPc accumulated in the LLC tumor tissue at 24 h after administration via DPPC liposomes (0.85 ± 0.15 $\mu g\ g^{-1}$ of tissue versus 2.16 ± 0.63 $\mu g\ g^{-1}$ recovered after injection in Cremophor EL), there is very little difference in the extent and rate of tumor response to the PDT in the two sets of experimental conditions. This is clearly demonstrated by the data reported in Fig. 2 and Table 2. Thus, it appears that the intratumoral concentration of SiPc reached by DPPC liposome delivery is sufficient to warrant an efficient necrosis of the neoplastic lesion. At the same time, DPPC liposome-administered SiPc did not appear to induce a more extensive skin photosensitization than Cremophor-delivered SiPc. As a consequence, DPPC liposomes represent a more convenient delivery system for SiPc, at least in our experimental animal model. As a matter of fact, the use of DPPC liposomes brings about a smaller uptake and a faster clearance of the phthalocyanine from the brain. This should minimize the risk of any toxic effect of SiPc at the level of the central nervous system: the occurrence of such potentially dangereous side effect is often overlooked in studies on the pharmacokinetic properties of PDT agents. The possibility of utilizing liposomal formulations for the systemic injection of hydrophobic porphyrin derivatives is supported by the recent progress in liposome technology, which allowed the development of liposome–drug associations with a prolonged shelf-life [14].

Studies are presently in progress in our laboratories with the aim of elucidating the mechanism involved in the SiPc-photosensitized necrosis of the LLC tumor.

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