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Effect of the delivery system on the biodistribution of Ge(IV) octabutoxy-phthalocyanines in tumour-bearing mice

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Abstract

The pharmacokinetic properties of the Ge(IV)-octabutoxy-phthalocyanines (GePc) with two axially ligated triethyl-siloxy (GePcEt) or trihexyl-siloxy (GePcHex) chains were studied in BALB/C mice bearing a transplanted MS-2 fibrosarcoma. The GePcs were delivered to mice after incorporation into unilamellar liposomes of dipalmitoyl phosphatidylcholine (DPPC) or in an emulsion of Cremophor-EL. The Cremophor delivered GePcs were cleared from the blood circulation at a much slower rate than the liposome-delivered GePcs. At the same time, Cremophor induced a slower and reduced uptake of the GePcs in the liver and spleen while it greatly enhanced the uptake in the tumour as compared to liposomes. Maximum tumour uptake was observed at 24 h post-injection and was equivalent to 0.67 and 0.50 nmol/g, respectively, for the Cremophor delivered GePcHex and GePcEt. The corresponding values for the liposome-delivered drugs were approximately one fourth of that observed with Cremophor.

Keywords: Phthalocyanines; Photodynamic therapy; Liposomes; Cremophor-EL; Tumour

1. Introduction

Some second generation photosensitizers proposed for the photodynamic therapy of tumours (PDT) are water-insoluble compounds and require a lipid-based delivery system for their systemic injection. In this connection, unilamellar liposomes of various phospholipidic compositions have been widely used for the administration of highly hydrophobic phthalocyanines to tumour-bearing animals [1–3]. Similarly, emulsions of the ethoxy-castor oil Cremophor EL or inclusion complexes of γ-cyclodextrin were utilized for the delivery of other porphyrin-derived macrocyclic compounds such as purpurins [4,5]. In general the photosensitizers associated with these delivery systems accumulate in the tumour with a reasonably high degree of selectivity, probably because of their preferential transport in the bloodstream by

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lipoproteins [6]. One lipoprotein family, namely low-density lipoproteins (LDL), appears to play a major role in the transport of hydrophobic sensitizers to tumour tissues by enhancing their selective uptake through a receptor mediated endocytotic mechanism [7,8]. Since many types of neoplastic cells express a higher LDL receptor activity than normal cells [9] one should expect a correlation between the LDL binding of the photosensitizer and its tumour uptake. However, other serum proteins and transport modalities have been also proposed as determinants of the efficiency of photosensitizer uptake by tumours [10]. This hypothesis receives some support from recent findings obtained in our laboratory. Thus, a $(trihexyl\text{-}siloxy)_2$ Ge(IV) octabutoxy-phthalocyanine administered to tumour-bearing mice in unilamellar liposomes of dipalmitoyl-phosphatidycholine (DPPC) or a Cremophor-EL emulsion distributes among serum lipoproteins quite similarly but it is accumulated by the tumour with a 4-fold higher efficiency after delivery via Cremophor. On the contrary, the Cremophor emulsion induces a 3-fold increase of the LDL binding on a percent basis for the decyl phthalocyanine analogue and a corresponding 3-fold larger tumour uptake. These observations suggest that the delivery system can greatly affect the uptake of hydrophobic photosensitizers in the tumour. In order to shed more light on this topic we performed more detailed pharmacokinetic studies with two Ge(IV)-octabutoxy phthalocyanines bearing two trialkyl-syloxy ligands in the fifth and sixth coordination position of the metal ion and delivered to tumour-bearing animals in DPPC liposomes or in the Cremophor emulsion. In particular, we correlated the kinetics of uptake of the photosensitizer in the tumour with that in liver and spleen and with the clearance from the plasma.

2. Materials and methods

2.1. Phthalocyanines

The GePcEt and the GePcHex were synthesized according to the procedure described by Rihter et al. [12]. The incorporation of the GePcs into unilamellar liposomes of DPPC (Sigma, St. Louis, MO) was performed according to the method of Kremer et al. [13]. The same GePcs were solubilized in Cremophor EL (Sigma) following the original procedure of Morgan et al. [14]. Before administration to the animals the GePc concentration in the liposomal or Cremophor suspension was determined spectrophotometrically using extinction coefficients at 760 nm of $2.46 \times 10^5\ M^{-1}\ cm^{-1}$ and $2.33 \times 10^5\ M^{-1}\ cm^{-1}$, respectively, for GePcEt and GePcHex in tetrahydrofuran.

2.2. Animals and tumour

Female BALB/C mice were purchased from Charles River (Como, Italy) and kept in standard cages with free access to tap water and standard dietary feed. Animal care was performed according to the guidelines established by the Italian Committee for Experimental Animals. The MS-2 fibrosarcoma was intramuscularly implanted in the right hind leg of the mice by injection of 2×10^5 cells suspended in 0.2 ml of sterile physiological solution. The tumour underwent no spontaneous regression and reached a diameter of ~0.7 cm at 7–8 days after implantation. At this time the mice were utilized for the pharmacokinetic studies.

2.3. Pharmacokinetic studies

The tumour-bearing mice were i.v.-injected with Ge Pc in DPPC liposomes or in Cremophor emulsion at a dose of 0.35 μmol/kg body weight. Initial plasma concentration of Cremophor was about 5.4 mg/ml. At time intervals between 3 h and 7 days after drug administration the mice were sacrificed by exposure to ether vapours. The blood, the tumour as well as liver, spleen, kidney, muscle and skin were removed, and frozen until the analysis of the GePc content was performed following the procedure previously described [15]. The tissues (~200 mg) were homogenized with 2% SDS (3 ml), the homogenate was diluted 3-fold with tetrahydrofuran and centrifuged at 3000 rev./min in order to obtain a transparent extract. The plasma (0.05 ml) obtained after blood centrifugation was diluted by addition of 2% SDS (0.7 ml) and tetrahydrofuran (1.5 ml) and centrifuged as specified for the tissue extracts. The tissue and plasma extracts were analyzed at the spectrophotofluorimeter (Perkin-Elmer MPF4, excitation at 690 nm, emission measured in the 720–880

nm range) and the measured fluorescence intensity was converted into GePc concentration by interpolation with a calibration plot.

3. Results

The analysis of the GePcEt and GePcHex concentration in the plasma of the tumour bearing mice showed that the DPPC liposome-delivered drugs are cleared from the blood more rapidly than the Cremophor-delivered drugs (Fig. 1). At 3 h post-injection both liposome-delivered GePcs are almost completely cleared from plasma while ~35% and 45% of the Cremophor-delivered GePcEt and GePcHex, respectively, are still in the plasma. The GePc concentration in the plasma at time 0 was estimated by assuming the blood weight to be equal to 5.8% of the mouse body weight and the haematocrit equivalent to 41.5% of the whole blood [16]. The uptake and release of the liposome- and Cremophor-delivered GePcs in the tumour are shown in Fig. 2. The maximum drug concentration was found at 24 h post-injection with both delivery systems. However, the Cremophor emulsion remarkably enhances the tumour uptake of the GePcs, as shown by the larger recovery (~4-fold) found at all time points examined. The delivery system also affected the rate and amount of GePcs accumulated by liver

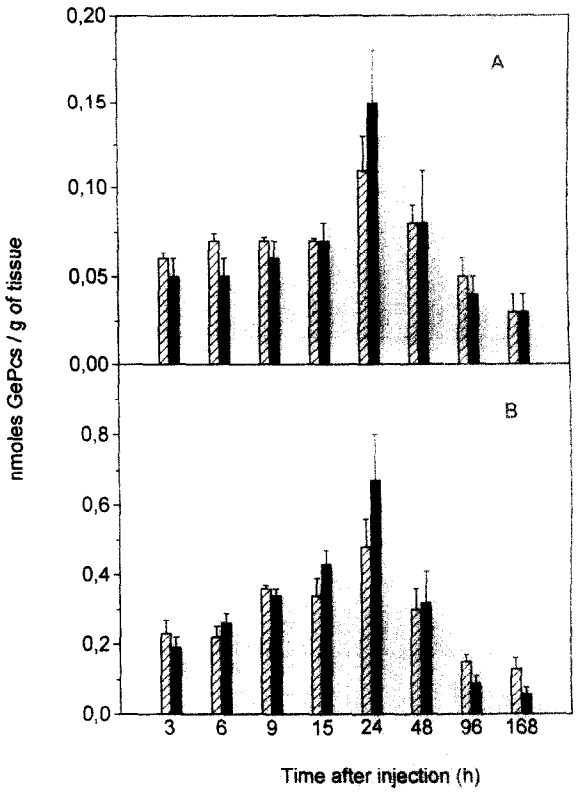

Fig. 2. Recoveries of GePcEt (▨) and GePcHex (■) from the tumour, at different times after the administration of 0.35 μmoles/kg in DPPC liposomes (A) or in Cremophor EL emulsion (B).

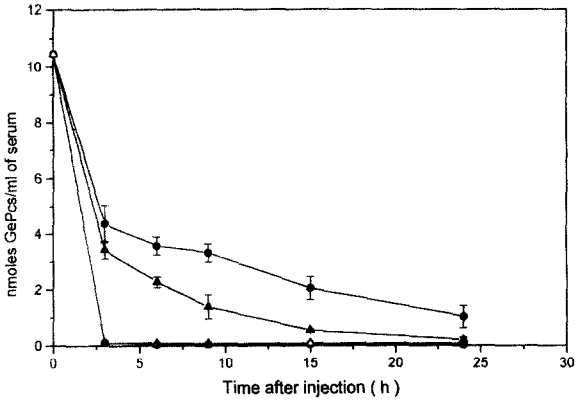

Fig. 1. Time-dependence of GePc concentration in the plasma of tumour-bearing mice after the i.v. injection of 0.35 μmol/kg GePcEt in DPPC liposomes (Δ) or in Cremophor-EL emulsion (▲) and GePcHex in DPPC liposomes (○) or Cremophor EL emulsion (●).

and spleen. For the post-injection time intervals studied, maximal GePc concentrations in both tissues were reached already at 3 h upon DPPC liposome delivery with a slow decline at time intervals longer than 24 h (Figs. 3A and 4A). On the other hand, Cremophor-delivered drugs were accumulated at a remarkably lower rate and in smaller amounts, with maximal levels at 24 h post-injection (Figs. 3B and 4B). A particularly low liver uptake is observed for GePc-Hex at short time intervals (Fig. 3B); however, this derivative is cleared at a slower rate from spleen (Fig. 4B). The analysis of the GePc content in the kidney, muscle and skin, at different times after administration showed very low drug recoveries. The values and post-injection time of the maximal recoveries for the two GePcs are reported in Table 1.

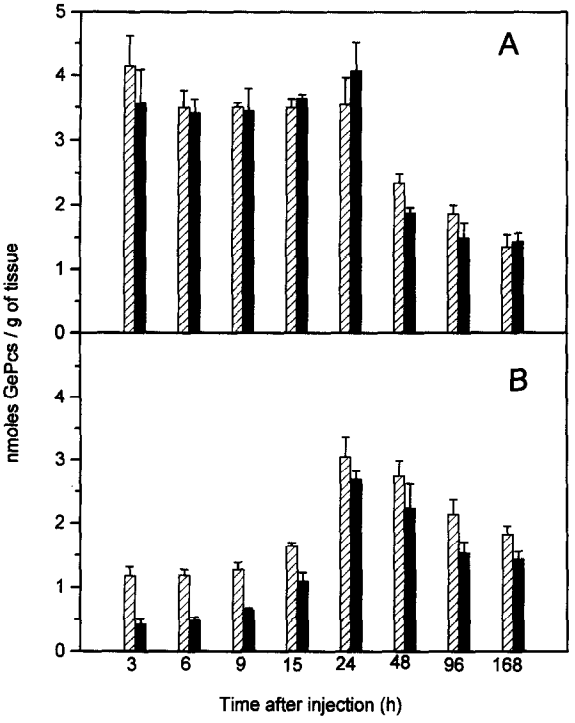

Fig. 3. Recoveries of GePcEt (▨) and GePcHex (■) from the liver of tumour bearing mice, at different times after the administration of 0.35 μmol/kg in DPPC liposomes (A) or in Cremophor-EL emulsion (B).

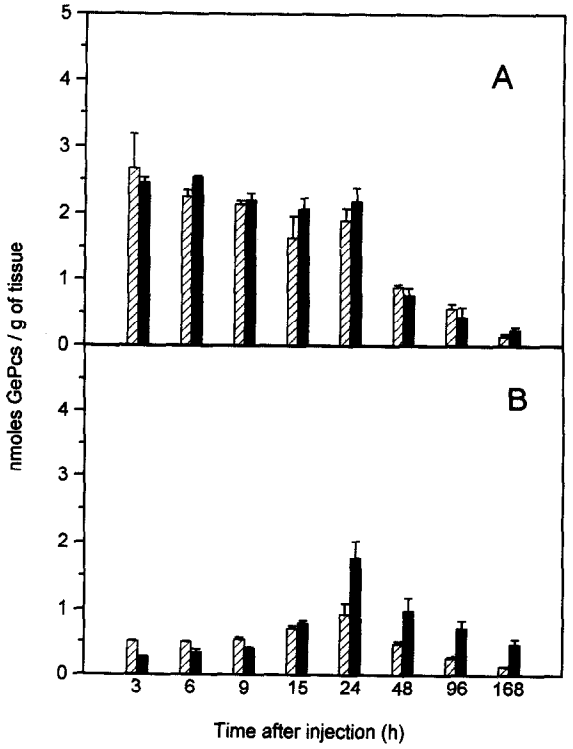

Fig. 4. Recoveries of GePcEt (▨) and GePcHex (■) from the spleen of tumour-bearing mice at different times after the administration of 0.35 μmol/kg in DPPC liposomes (A) or in Cremophor-EL emulsion (B).

4. Discussion

Both GePcEt and GePcHex delivered via DPPC liposomes or Cremophor emulsions show a very similar pharmacokinetic behaviour, and in particular a good selectivity of tumour targeting. Very low amounts of both phthalocyanines are recovered from healthy tissues, such as skin and muscle (Table 1), which should minimize the risk of photosensitized damage to such tissues. The kidneys accumulate a relatively large amount of Cremophor-delivered GePcs at short times, however a rapid drug clearance is observed with recoveries below 0.1 μg/g at 1 week. On the other hand, the GePcs administered to tumour-bearing mice, after incorporation into DPPC liposomes,

Table 1
Maximal recoveries (nmoles/g tissue) of the GePcs from selected normal tissues of tumour-bearing BALB/C mice after administration of 0.35 μmol/kg in DPPC liposomes or Cremophor emulsion. The reported values are determined at 24 h unless otherwise specified

Drug	Muscle		Skin		Kidney	
	DPPC	Cremophor	DPPC	Cremophor	DPPC	Cremophor
GePc-Et	0.05 ± 0.00	0.07 ± 0.02	0.05 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.25[a] ± 0.02
GePc-Hex	0.03 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.32[a] ± 0.04

[a]Values determined at 3 h.

show a fast clearance from the blood circulation and a corresponding fast uptake in the liver and spleen (see Figs. 1, 3A and 4A). This behaviour is typical of drugs delivered in vivo in association with conventional liposomes; i.e. liposomes composed of various phospholipids and cholesterol with no specific stabilizing components. In fact, it has been shown that many types of antiparasitic, antifungal and anticancer drugs, delivered via liposomes, are removed from the circulation within a few minutes, by the macrophages of the components of the reticuloendothelial system (RES) [17–19]. This has limited the prospect of using the liposomes as an in vivo delivery system for transporting drugs to sites different from those of the RES. In spite of these limitations, liposomes have been shown to be useful for the delivery of hydrophobic photosensitizers acting as photodynamic agents in the PDT of tumours [1,3]. In the case of moderately hydrophobic photosensitizers, such as hematoporphyrin, liposomes guarantee a higher and more selective tumour targeting as compared to the free drug [20]. The latter findings were explained by the transfer of about 70% of the injected drug to serum lipoproteins that, in turn, transport the bound drug to tumour cells [8]. The data obtained in the present investigation suggest that although GePcEt and GePcHex are bound in relatively large amounts by LDL, the high tumour targeting observed in the case of the Cremophor delivered GePcs (Fig. 2) is related also to the slower clearance from the plasma (Fig. 1) and the parallel slower uptake in liver (Fig. 3) and spleen (Fig. 4). The slow and reduced uptake in liver and spleen could enhance the chances for drug localization in sites different from those of the RES and especially in the tumour. The slow clearance from the circulation of the Cremophor-delivered GePcs can be due to the high stability of the lipidic droplets of this particular emulsion in the presence of serum lipoproteins (Polo et al., unpublished observations) and/or to a more difficult diffusion of the droplets through the fenestrated capillaries of the RES organs. It has been reported that liposome formulations with a prolonged circulation time in the blood enhance the antitumour activity of doxorubicin and epirubicin as compared to conventional liposome formulations [21]. It must be noted that Cremophor, especially in the form of the small lipid droplets utilized by us, shows a strong tendency to interact with lipoproteins, including LDL [22–25]. Thus, the circulating drug would be either associated with modified lipoproteins or embedded into the residual free Cremophor particles; this circumstance could appreciably affect the serum clearance and tissue uptake of the photosensitizer relative to the liosome delivered photosensitizer. Hence it appears that the delivery system plays a major role in modulating the efficiency and selectivity of tumour targeting by photosensitizers, as it has also been proposed by Woodburn and Kessel [22]. The Cremophor emulsion seems to be a more convenient delivery system than DPPC liposomes since it guarantees the persistence of high drug concentrations in the blood circulation. In this connection, it may be important to test other long circulating delivery systems such as sterically stabilized liposomes [11]. In conclusion, the efficacy of the PDT treatment can be improved by combining appropriate photosensitizers and delivery systems.

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