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Liposome-delivered ^{131}I-labelled Zn(II)-phthalocyanine as a radiodiagnostic agent for tumours

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Abstract

^{131}I-Zn(II)-phthalocyanine (ZnPc) incorporated into unilamellar liposomes has been systemically injected to mice bearing a transplanted MS-2 fibrosarcoma. Biodistribution studies show that the pharmacokinetic behaviour of ^{131}I-ZnPc is very similar to that defined for the parent molecule ZnPc including a serum half-life of ca. 12 h, a high recovery from liver and spleen and minimal accumulation in kidney and brain The most important pharmacokinetic parameter is represented by the high tumour/muscle ratio of ^{131}I-ZnPc concentration (ca. 9 at 24 h post-injection). These results suggest the possible use of the radiolabelled derivative for a real-time non-invasive monitoring of the ZnPc concentration in the tumour and peritumoural tissue during photodynamic therapy.

Keywords: Tumours; Phthalocyanine; Liposomes; Radiodiagnostic agents; Gamma-camera

1. Introduction

Several porphyrins and phthalocyanines are known to possess a good efficiency and some selectivity of tumour targeting [1–3]. In particular, one derivative of hematoporphyrin, PhotoErin II, has now been approved for photodynamic therapy (PDT) of specific tumour types, while some phthalocyanines and in particular Zn(II)-phthalocyanine are now being introduced into phase I/II trials. Phthalocyanines show favourable physico-chemical and spectroscopic properties for acting as photosensitizing agents in vivo [4,5].

Biodistribution and tumour-photosensitization studies with liposome-incorporated Zn(II)phthalocyanine [6,7] pointed out that this compound is phototherapeutically active at doses as low as 0.15 mg/kg body weight with an enhanced selectivity of tumour accumulation.

Since most photosensitizing agents are endowed with a significant fluorescence yield, Photofrin has been also proposed for in vivo photodiagnosis of neoplastic lesions. The technique has been used for detection of neoplasias localized in several organs including lung, urinary bladder, colon, stomach and brain [8–10].

However, in the present state of development, diagnostic modalities based on fluorescence measure-

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ments in vivo present some limitations: (1) the fluorescence quantum yield can be influenced by the aggregation of the porphyrin in cells or tissues; (2) the microenvironment of the dye can also affect the fluorescence properties; (3) several experimental artifacts can induce false positives or negatives [11].

In principle, these limitations could be overcome, at least in part, by the use of radiolabelled photosensitizers, since radiodiagnostic measurements are much less sensitive to the aggregation of the dye and the nature of the microenvironment [12]. Thus, porphyrin or phthalocyanine derivatives bearing either a radioisotope of the centrally coordinated metal ion [13] or specifically radiolabelled atoms in the macrocycle [14] have been proposed with generally encouraging results.

In this paper we propose the use of ZnPc labelled with ^{131}I for early diagnosis of tumours. For this aim we studied the pharmacokinetic behaviour of the ^{131}I-ZnPc incorporated into unilamellar liposomes of D,L-α-dipalmitoyl-phosphatidylcholine (DPPC) in Balb/c mice affected by a MS-2 fibrosarcoma. This tumour model had been previously used for extensive PDT studies with unlabelled ZnPc [6,7].

2. Materials and methods

2.1. Animals and tumours

Female Balb/c mice, 20–22 g body weight, were obtained from Charles River (Como, Italy). The mice were kept in standard cages with free access to normal dietary chow and tap water. The MS-2 fibrosarcoma has been originally supplied by Istituto Nazionale Tumori (Milan, Italy).

For tumour implantation 2×10^5 cells in 0.2 ml of sterile physiological solution were intramuscularly injected into the right hind leg of the mouse. All the experiments were started on the eighth day after tumour implantation, when the external diameter of the neoplasia was ca. 0.8 cm and no detectable spontaneous tumour necrosis had generally occurred. When necessary, mice were anaesthetised by i.p. injection of Ketalar (150 mg/kg). In all cases, animal care was performed according to the guidelines established by the Italian Committee for Experiments on Animals.

2.2. Chemicals

Zn(II)-phthalocyanine was supplied by Ciba-Geigy (Basel, Switzerland) and used as received. Chemical analysis of the compound showed a degree of purity of 99%. Na ^{131}I (185 MBq) was supplied by Nordion Europe S.A. (Belgium). D,L-α-Dipalmitoyl-phosphatidylcholine (DPPC) was a product of Sigma Chemical Co. All other chemicals and solvents were analytical grade reagents.

2.3. Radioiodination of Zn(ii)-phthalocyanine

Typically, 0.02 ml of 13.5 nM Na ^{131}I (185 MBq) in 0.1 M NaOH solution were introduced into a reactivial and the water removed by azeotropic distillation under a nitrogen stream. Then 0.1 mg of Zn(II)-phthalocyanine in 0.1 ml of anhydrous dimethylformamide (DMF) and 0.05 mg of chloramine T were added to the vial and the reaction mixture was heated to 100°C for 15 min. Electrophoretic studies on labelling kinetics show that labelling yields of 60–70% are obtained after 15–20 min at 100°C. The labelling reaction was stopped by adding 0.1 mg of $Na_2S_2O_4$ and the product was purified by anion exchange chromatography to remove any unreacted iodine. The ^{131}I-ZnPc was eluted as a single peak: although no specific characterization of the compound was performed, it appears reasonable to assume that the ZnPc was almost exclusively monoiodinated since the phthalocyanine/Na-^{131}I molar excess in the reaction mixture was about 630.

2.4. Preparation of the injectable formulation

^{131}I-Zn(II)-phthalocyanine was incorporated into small unilamellar vesicles of DPPC following the procedure described by Valduga et al. [15]. Before incorporation into the lipid matrix DMF was removed by lyophilization and the residue solubilized in anhydrous pyridine.

For these experiments, liposomes were prepared in 0.9% aqueous NaCl and dialyzed for 3 h against 250 ml of saline with a change after the first hour. The incorporation yield of ^{131}I-Zn(II)phthalocyanine into unilamellar liposomes of DPPC was 30–35%.

'Empty' liposomes devoid of ZnPc were prepared by introducing 0.02 ml of Na-^{131}I (185 MBq) into a

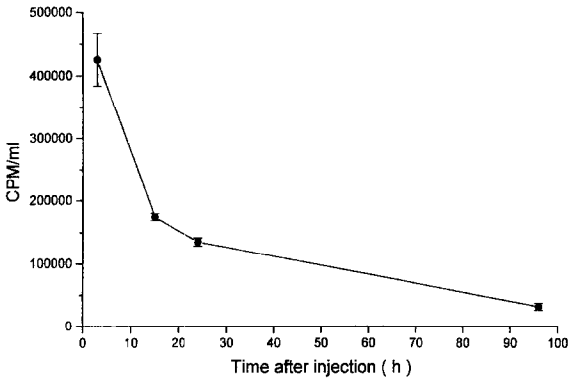

Fig. 1. Effect of time on the clearance of ^{131}I-ZnPc from serum of Balb/c mice bearing a MS-2 fibrosarcoma. The mice were injected in the tail vein with 0.096 MBq/g ^{131}I-ZnPc (0.43 mg/kg). Average of at least three independently analyzed mice at each time.

reacti-vial and removing water by azeotropic distillation under a nitrogen stream. The residue was solubilized in anhydrous pyridine and incorporated into small unilamellar vesicles of DPPC.

2.5. *Pharmocokinetic studies*

Balb/c mice bearing the MS-2 fibrosarcoma were injected in the tail vein with ^{131}I-ZnPc at doses between 0.024 and 0.096 MBq/g.

At predetermined times up to 24 h after injection the whole bodies of the mice were scanned with a Gamma Camera General Electric Stargem in order to measure the radioactivity levels at different anatomical sites. Then the mice were sacrificed by prolonged exposure to ether vapours; the blood, tumour and selected normal tissues (muscle, skin, lung, liver, spleen, kidney, brain) were rapidly taken. The blood was centrifuged to remove the erythrocytes and the radioactivity in the plasma (100 μl) was analyzed. Moreover, tissues were washed with physiological solution, weighed, introduced into RIA test tubes and centrifuged for 10 min at 2000 $\times$ g. The radioactivity was collected for 1 min by a gamma counter (Packard Selektronik Design). A different group of mice was injected as above described, sacrificed at 3 h, 15 h, 24 h, 48 h and 96 h after injection (three mice at each time), and the radioactivity in plasma and tissues homogenates was determined.

3. Results

The pharmacokinetic behaviour of i.v.-injected ^{131}I-ZnPc (0.096 MBq/g) incorporated into unilamellar liposomes of DPPC was examined. As shown in Fig. 1, ^{131}I-ZnPc is almost completely eliminated from mouse serum within 96 h; the clearance rate is particularly fast during the initial 15 h after administration.

The time dependence of ^{131}I-ZnPc distribution in tumour and selected normal tissues is shown in Table 1. The recovery of ^{131}I-ZnPc is very high in the liver and spleen, as expected since the components of the reticuloendothelial system exhibit a high affinity for systemically injected lipid-type particles [16]. Moreover, hydrophobic photosensitizers are largely eliminated from the organism via the bile-gut pathway [17]. This conclusion is confirmed by the comparatively low levels of phthalocyanine accumulated in the kidneys.

Table 1

Recoveries (CPM/mg of tissue) of ^{131}I-ZnPc from selected tissues of tumour-bearing mice at different times after injection of 0.096 MBq/g ^{131}I-ZnPc in DPPC liposomes

Tissue	Time				
	3 h	15 h	24 h	48 h	96 h
Liver	984.2 ± 15.7	871.1 ± 146.8	1043.7 ± 72.5	849.1 ± 90.3	831.9 ± 52.5
Spleen	600.9 ± 8.1	503.7 ± 46.8	635.1 ± 65.8	549.7 ± 96.2	413.0 ± 42.7
Kidney	156.9 ± 9.7	84.4 ± 8.4	77.3 ± 11.2	59.4 ± 7.5	61.6 ± 16.8
Tumour	140.3 ± 38.6	182.7 ± 16.0	212.9 ± 8.6	180.7 ± 31.4	162.2 ± 19.5
Muscle	41.1 ± 3.6	22.4 ± 9.3	23.7 ± 2.0	22.3 ± 0.9	15.8 ± 2.5
Lung	168.3 ± 5.6	76.2 ± 31.4	83.8 ± 12.4	73.4 ± 9.9	52.1 ± 6.9
Brain	11.7 ± 0.1	5.2 ± 1.5	4.3 ± 0.6	3.4 ± 0.7	2.0 ± 0.4

Average of three independently analyzed mice ± SD.

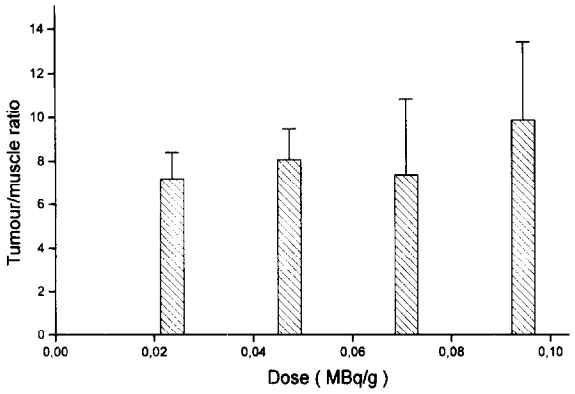

Fig. 2. Ratio between the tumour/muscle radioactivity levels of ^{131}I-ZnPc at different concentrations at 24 h after injection of ^{131}I-ZnPc in DPPC liposomes. Averaged ratios for three independently analyzed mice ± SD.

On the other hand, minimal amounts of ^{131}I-ZnPc are recovered from brain, hence any toxic effects of the drug on the central nervous system are unlike-ly.

The most important pharmacokinetic parameter is represented by the difference in ^{131}I-ZnPc concentration between tumour and muscle, which is the peritumoural tissue in our animal model, thus indicating the level of selectivity of tumour targeting. While the maximum accumulation in tumour is observed at 24 h, small amounts of phthalocyanine are recovered from muscle at all times (Table 1). The tumour-to-muscle concentration ratio can be taken as an index of the selectivity of human tumour targeting by ^{131}I-ZnPc. Such ratio is between 8–9 already at 15 h after injection and remains at an essentially constant level throughout the time interval studied by us.

Similar experiments were performed as a function of the injected ^{131}I-ZnPc dose. As one can see in Fig. 2, the tumour/muscle ratio of phthalocyanine concentration at 24 h after administration is affected to a minor extent by the injected dose.

The pharmacokinetic results obtained with empty liposomes show a time-dependent pattern of ^{131}I radioactivity similar to that observed for the phthalocyanine, namely an increase of the tumour/muscle ratio up to 24 h.

4. Discussion

The data obtained in the present investigation clearly indicate that ^{131}I-labelled ZnPc is an efficient and selective tumour localizer. The pharmacokinetic properties of this phthalocyanine show several similarities with those previously defined for the parent molecule ZnPc in the same animal model [6,7]. Such analogies include: (1) the apparently polyphasic clearance from the serum with almost 80% of the initial radioactivity disappearing within the initial 24 h post-injection; (2) the relatively low rate of uptake by the tumour as compared to several normal tissues: the maximal concentration of phthalocyanine in the tumour is reached after ca. 24 h; (3) the large amount accumulated by liver and spleen, which is followed by a relatively slow elimination at least during the initial 4 days; this suggests that the most important mechanism for ^{131}I-ZnPc elimination from the organism occurs via the bile-gut pathway with only minor amounts cleared via the kidneys.

The main differences between ^{131}I-ZnPc and ZnPc are represented by the greater accumulation of the former phthalocyanine in the liver at short post-injection times (ca. seven-fold larger concentration than in the tumour) and, most of all, by its almost two-fold higher selectivity of tumour targeting, as shown by the tumour/muscle ratios of phthalocyanine recovery. This behaviour can be explained, at least in part, by the increased hydrophobicity imparted to the tetraazaisoindole macrocycle of the phthalocyanine by the iodine substituents, since hydrophobic photosensitizers usually exhibit a greater affinity for tumour tissues [18].

ZnPc has been repeatedly observed to be an efficient tumour-targeting dye [6,7,19,20]; therefore, our findings strongly support the possibility of using ^{131}I-ZnPc as a radiodiagnostic agent for tumours which could overcome at least some of the drawbacks associated with the fluorescence diagnostic technique (see Section 1). Our in vivo analysis of the radioactivity distribution in the whole animals (data not shown) points out that at all time intervals examined by us the radioactivity is strictly confined to liver and tumour with no general delocalization throughout the organism; hence, it is unlikely that ^{131}I is dissociated from the phthalocyanine to any significant extent. ZnPc itself is eliminated in the faeces with

no chemical alteration [6]. Thus, tumour diagnosis based on radioemission by ZnPc-associated ^{131}I should yield very reliable information.

Moreover, since the kinetics of tumour uptake and clearance are quite similar for ^{131}I-ZnPc and ZnPc (a phototherapeutic agent), one could envisage the use of the radiolabelled derivative for a real-time non-invasive monitoring of the ZnPc concentration in the tumour and peritumoural tissue, in order to identify the most convenient post-injection time interval for performing the PDT treatment. In this way, uncertainties due to individual variability of the photosensitizer biodistribution should be minimized. Experiments aimed at the definition of this possibility are presently in progress in our laboratories.

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