Pharmacokinetic studies with zinc(II)-phthalocyanine in tumour-bearing mice

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Summary Zinc(II)-phthalocyanine (Zn-Pc) incorporated into unilamellar liposomes of dipalmitoylphosphatidylcholine has been injected intraperitoneally (0.5 mg kg⁻¹) to BALB/c mice bearing a transplanted MS-2 fibrosarcoma. The drug is specifically transported by serum lipoproteins and cleared from the serum via the bile-gut pathway in a biphasic process: ~60% of Zn-Pc is eliminated with a serum half-life of ~9 hours, while the remaining aliquot is eliminated at a very slow rate. Several normal tissues take up the drug within 3 hours after administration but release it almost completely after 24–48 hours. On the other hand, the tumour shows a maximum concentration of Zn-Pc (~0.6 µg g⁻¹ of tissue) after 18–24 hours; at this time, the ratio between the Zn-Pc levels in the tumour and the muscle (which represents the surrounding normal tissue) is ~7.5. The results are discussed in terms of a possible use of Zn-Pc as a photosensitizer in the photodynamic therapy of tumours.

Haemoporphyrin and haematoporphyrin derivative (HpD) are presently used as sensitizers for the specific destruction of tumours by photodynamic therapy (PDT) (Moan, 1986; Spikes & Jori, 1987). Although the technique is being applied for the treatment of a variety of neoplasias at both the experimental and clinical level (Dougherty, 1984), PDT is facing some problems and/or limitations. Thus, HpD as it is prepared from Hp (Lipson et al., 1961) is a complex mixture of porphyrins, whose composition is often not reproduced in different preparations and may change as a function of various experimental parameters (Bonnett et al., 1981; Dougherty, 1983). So far, the most active components of HpD, which have been reported to possess an ether (Dougherty, 1985) or ester (Kessel, 1985) structure, have not been isolated in a pure form. Moreover, PDT is usually performed by irradiation with 620–630 nm light; unfortunately, the molar absorptivity of Hp or HpD in this wavelength range is very low (<10⁶ M⁻¹ cm⁻¹).

Phthalocyanines, which are structurally similar to porphyrins, can potentially overcome some limitations of PDT; in particular, they exhibit a strong absorption (ε=10⁵ M⁻¹ cm⁻¹) in the 680–700 nm region i.e., in correspondence of light wavelengths endowed with a high penetration power into biological tissues. The ability of phthalocyanines and some of their metal derivatives to act as efficient photosensitizers of simple biological substrates (Spikes & Bommer, 1986) and cultured cells (Ben-Hur & Rosenthal, 1985; Chan et al., 1986) has been documented. Finally, Rousseau et al. (1985) have reported that tetrasulphophthalocyanines (TSPc) can accumulate in a mammary adenocarcinoma in rats.

While tetrasulphophthalocyanines are suitable for in vivo studies because of their high water-solubility, serious problems may arise owing to the low degree of purity. Actually, the presently adopted procedures for sulfonation of phthalocyanines lead to mixtures of mono-, di-, tri- and tetra-sulphonated compounds (Linstad & Weiss, 1950; Moser & Thomas, 1983).

In general, underivatized phthalocyanines can be obtained with a very high purity and display a good efficiency in the generation of activated oxygen species (Maillard et al., 1980; Wu et al., 1985). Such water-insoluble dyes can be transported in the bloodstream via unilamellar liposomes (Valduga et al., 1987); the latter carriers were shown to induce an efficient targeting of experimental tumours by water-insoluble porphyrins (Jori et al., 1983), possibly through the preferential delivery of the incorporated drug to serum lipoproteins (Jori, 1985). In this paper, we describe the pharmacokinetic behaviour of liposome-bound Zn-phthalocyanine injected into BALB/c mice bearing a transplanted fibrosarcoma.

Materials and methods

Chemicals

Zn²⁺-phthalocyanine (Zn-Pc) was supplied by Ciba-Geigy (Switzerland) and used without further purification. Sublimation of the sample under high vacuum showed a degree of purity of 97% (Valduga et al., 1987). Dipalmitoylphosphatidylcholine (DPPC), over 98% pure, was a product of Sigma Chemical Co.; sodium dodecylsulphate (SDS) and Sephacryl S-300 were purchased from Merck and Pharmacia, respectively.

Animals and tumour

Female mice of the BALB/c strain (20–25 g body wt) were obtained from Charles River (Como, Italy). The MS-2 fibrosarcoma was kindly supplied by Instituto Nazionale dei Tumori, Milan. It was implanted in the right hind leg of the mice by injection of 10⁵ cells suspended in 0.1 ml of PBS. The pharmacokinetic studies were begun at 8 days after tumour implantation, when its diameter was in the 0.7–1.0 cm range.

Pharmacokinetic studies

The Zn-Pc was injected i.p. into tumour-bearing mice after incorporation of the dye into small unilamellar liposomes of DPPC following the procedure described by Valduga et al. (1987): The Zn-Pc/DPPC ratio was 1:36 on a molar basis, as assessed from the weighed amount of phospholipid and absorbance measurements at 680 nm for Zn-Pc (ε=1.68 × 10⁶ M⁻¹ cm⁻¹ at 673 nm). The injected dose of Zn-Pc was 0.5 mg kg⁻¹ mouse body wt. At fixed times after Zn-Pc administration the mice were sacrificed and several tissues (tumour, muscle of the left hind leg, skin, liver, spleen, kidneys, lungs) were removed, washed with PBS and frozen until the analysis for Zn-Pc content was performed. Blood samples were collected from the same mice, centrifuged at 3,000 rpm for 15 min and the serum was analyzed for Zn-Pc content. The elimination pathway of Zn-Pc was studied by analysis of the faeces and urine collected from healthy Wistar albino rats, which had been injected with liposome-bound Zn-Pc at a dose of 0.5 mg kg⁻¹. Through-

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Received 13 April 1987; and in revised form, 16 June 1987.
out the experiments the rats were maintained in metabolic
cages with free access to standard dietary chow and the
faeces and urines were collected until 48 h after the adminis-
tration of Zn-Pc.

Recovery of Zn-Pc from tissue and serum specimens
Tissue samples (~200 mg of wet wt) were homogenized in
4 ml of 2% aqueous SDS as previously described (Jori et al.,
1983). The homogenate was magnetically stirred for 1 h at
room temperature. The suspension thus obtained was
centrifuged at 3,000 rpm for 10 min; the supernatant was
collected and its Zn-Pc content (µg drug ml⁻¹ solution) was
estimated by fluorescence measurements with a Perkin-Elmer
MPF4 apparatus. The results of Zn-Pc recovery were finally
referred to an identical weight of each tissue, i.e., 1 g. The
sample was placed in quartz cuvettes of 1 cm optical path and
its 600 nm-excited fluorescence was recorded in the 630–
740 nm spectral interval at a right angle to the incident light
beam; in this way, the contribution of scattered light could
be more easily subtracted. To minimize inner filter effects,
the absorbance of the analyzed solutions at the excitation
wavelength was kept below 0.1. The fluorescence intensity
data were converted into Zn-Pc concentration by interpo-
lation with a calibration plot built with known
amounts of Zn-Pc in 2% SDS; preliminary studies (Valduga
et al., 1987) have shown that under our experimental
conditions Zn-Pc is embedded in a monomeric state within
the surfactant micelles. In preliminary studies, the pellet
remaining after removal of the supernatant was resuspended
in 2% SDS (4 ml) and processed as above described; only
negligible amounts of Zn-Pc fluorescence were observed.

Serum samples were diluted with suitable volumes of 2%
aqueous SDS, so that the absorbance at 600 nm was lower
than 0.1. The fluorescence emission of Zn-Pc was then
determined as described above. The Zn-Pc fluorescence
obtained from tissue extracts was corrected for the
contribution of a 600 nm-excitable background fluorescence
as observed in tissue extracts from control mice.

Chromatographic studies
Serum samples were chromatographed on a column
(1.7 x 140 cm) of Sephacryl S-300, which had been
equilibrated with 0.01 M phosphate buffer at pH 7.4,
containing 0.15 M NaCl. The column was eluted at a flow-
rate of 26 ml h⁻¹ and 2.5 ml-fractions were collected. The
fraction collector was connected to a 2238 LKB UV-cord
and the protein content was continuously recorded by
monitoring the absorbance of the eluate at 280 nm. The
collected fractions were also analyzed for their Zn-Pc content
by measuring the intensity at 680 nm of the 600 nm-excited
fluorescence. This value was found to be proportional to the
integrated area of the whole fluorescence spectrum.

Results
The serum concentration of i.p.-injected Zn-Pc reaches a
maximum value at ~3 h after administration (Figure 1).
About 60% of Zn-Pc is eliminated with a half-life of ~9 h,
whereas the serum levels of the remaining drug undergo an
approximately exponential decrease at a low rate. Closely
similar behaviour has been reported for Hpd administered
to patients (Zalar et al., 1977). The rapid clearance of Zn-Pc
from the serum appears to occur almost exclusively via the
bile-gut pathway. Actually, analyses of faeces eliminated
from Wistar rats which had been maintained in metabolic
cages for 48 h after i.p. administration of the drug showed
the presence of a total amount of Zn-Pc ranging between 109
and 127 µg as compared to a total recovery range between
0.66 and 0.81 µg of Zn-Pc from the urine collected over the
same period of time. Similarly, Tomio et al. (1982) observed

![Figure 1](image1)

**Figure 1** Time-dependence of Zn-Pc concentration in the serum
of BALB/c mice bearing the MS-2 fibrosarcoma. The mice have
been injected with 0.5 mg kg⁻¹ of Zn-Pc incorporated into DPPC
liposomes.

![Figure 2](image2)

**Figure 2** Chromatogram of the serum of BALB/c mice 24 h
after the administration of liposomal Zn-Pc at a dose of
0.5 mg kg⁻¹. The chromatogram show the absorbance at 280 nm
(O—O) and the fluorescence emission at 680 nm (● — ●).

that at least 98% of the totally eliminated haematoporphyrin
from Wistar rats is recovered in the faeces.

Column chromatography of mouse serum on Sephacryl S-
300 allows us to separate three protein fractions (Figure 2).
Typically, at 24 h after i.p.-administration to tumour-bearing
mice, the Zn-Pc fluorescence is specifically associated with
the second protein peak. This peak appears to be mainly
constituted by the lipoprotein class as determined by the
estimation of its cholesterol and phospholipid content (such
analyses were carried out as described by Jori et al., 1984).
The specific association of Zn-Pc with this group of serum
proteins is independent of the time after administration:
chromatographic patterns qualitatively identical with those
shown in Figure 2 were obtained for sera taken at various
times between 1 h and 1 week after administration of Zn-Pc.

The maximum concentration of Zn-Pc in the serum
responds with the maximum uptake of the drug by
several normal tissues, including liver, kidneys, spleen, lungs
and muscle (Figures 3 and 4). In Figure 4 we show
comparatively the time-dependency of Zn-Pc recovery from
the MS-2 fibrosarcoma and the muscle, i.e., the surrounding
normal tissue. All the recovery data represent the average of
the values obtained by separate analyses of the tissues taken
from groups of at least three animals for each time, the
largest deviation from the reported values being 20%.

Discussion
Some water-soluble metal complexes of TSPc have been
shown to be accumulated in significant amounts by brain
tumours implanted into mice (Frigerio, 1962) or other kinds
of tumours in rats (see, for a recent review, Spikes, 1986). The problem of the selectivity of TSPc uptake by tumour tissues as compared with the tissue where the tumour growth has been addressed by Tralau et al. (1987): their results, although preliminary, would indicate a similar tumour-specificity of TSPc in comparison with HpD.

The data presented in this paper show that the water-insoluble Zn-Pc is also a good tumour-localizer, at least in our animal model. In particular, maximal tumour concentrations of Zn-Pc are found at ~18-24 h after i.p. administration of the drug, while after this time period most Zn-Pc has been released from normal tissues. Thus, at 24 h, the ratio of Zn-Pc content between the tumour and the muscle, i.e., the surrounding normal tissue, is ~7.5. One should remember, however, that the muscle does not represent the normal tissue where the MS-2 fibrosarcoma originates or grows.

Several indications suggest that TSPc and Zn-Pc are transported and delivered to tumours by at least partially different mechanisms. Thus i.v. injected TSPc are rapidly (1-3 h) accumulated by a fibrosarcoma implanted s.c. in the flank and completely disappear from the serum within 3 h (Tralau et al., 1987). It is likely that the latter dyes are mainly bound by serum albumin, as it has been observed for tetrasulphonated porphyrins (Kessel, 1987; Milanesi et al., 1987); albumin does not enter tumour cells, hence the associated drug is present in the vascular stroma of the neoplastic tissue at least for relatively short (24 h) periods of time after its administration. On the contrary, Zn-Pc is specifically transported by lipoproteins: this fact is not surprising in view of the highly lipophilic nature of the drug, as well as the tendency of the liposome carriers of Zn-Pc to fuse with the lipid matrix of lipoproteins and to release the entrapped drug to lipoproteins (Mayhew & Papahadjopoulos, 1983). Now, lipoproteins, especially LDL, preferentially interact with neoplastic and endothelial cells of tumour tissues through a receptor-mediated endocytosis, so that the drug is delivered from inside the cells (Goldstein et al., 1979; Netland et al., 1985). The high efficiency of Zn-Pc binding by lipoproteins can thus be correlated with the high amounts of drug accumulated by the tumour tissue (>0.6 μg g⁻¹ tissue) in spite of the low injected doses, i.e., 0.5 mg kg⁻¹ body wt. The above considerations may explain the different pharmacokinetic behaviour between Zn-Pc and TSPc, although both types of phthalocyanines can act as good tumour-localizing agents. Certainly, the control of such a behaviour has a critical importance for achieving a satisfactory degree of selectivity in the targeting of tumour tissues by photosensitizing agents (Jori, 1985).

In this connection, Zn-Pc has the distinct advantage over HpD and photosfrin II of a more homogeneous distribution among serum proteins. It has been shown that both haematoporphyrin (Jori et al., 1984) and HpD (Kessel, 1987) are transported by at least three different classes of serum proteins; this fact may explain the heterogeneous and time-dependent distribution of these porphyrins in tumour tissues (Kessel, 1986).

On the other hand, detectable amounts of HpD and Zn-Pc persist in the serum for some weeks after their administration. This circumstance has been claimed (Zalar et al., 1977) to be correlated with the prolonged skin photosensitivity, which represents one major side effect of the photodynamic therapy of tumours.

In order to obtain some information on this point, we estimated the skin concentration of Zn-Pc in mice at selected times. Although there was some degree of individual variability of the recovery data, in no case was the skin concentration of Zn-Pc between 1 h and 168 h after administration found to be >0.1 μg g⁻¹ tissue. Therefore, one would not expect a significant level of skin photosensitization by Zn-Pc. The relatively inefficient accumulation of Zn-Pc by mouse skin may again be a consequence of the mechanisms involved in the transport of this drug in the bloodstream. We have observed that, upon in vitro incubation of human serum with Zn-Pc incorporated into DPPC liposomes, a selective association of the phthalocyanine with lipoproteins takes place: at least 70% of the drug is bound by HDL (Reddi et al., unpublished results). HDL are known to be responsible for the prolonged serum persistence of a major fraction of i.v. injected haematoporphyrin (Barel et al., 1986).

In summary, the present findings encourage us to test the efficiency of liposome-carried Zn-Pc as a phototherapeutic agent: although the actual clinical use of this drug requires thorough toxicological studies. Experiments aimed at assessing the optimal parameters for photodynamic therapy of tumours with Zn-Pc are in progress in our laboratory.

This work received financial support in part from Consiglio Nazionale delle Ricerche (Italy), under the special project ‘Oncologia’, contract No. 86.00451.44, and in part from Teclas (Laser Technologies, Lugano, Switzerland). One of us (RB) is recipient of a fellowship given by Teclas.

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